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Colicinogeny and Related Phenomena

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INTRODUCTION

Analogies often provide useful models for investigating new phenomena. The inhibition of bacteria by closely related species was well documented in the nineteenth century and in the early years of this century (96, 153, 154). However, progress in understanding the nature of bacteriocins stems from the investigations of Gratia (207, 208). Gratia's work on the inhibition of *Escherichia coli* strain ϕ by a substance released from *E. coli* strain V was conducted very largely in relation to analogies provided by the phenomena of bacteriophage, which had recently been described by Twort and d'Herelle, and lysogeny, which had been described by Lisbonne and Carrère. A network of analogies has linked the study of colicins with other aspects of microbial genetics and molecular biology, and particularly with bacteriophages, to the present day.

The initial studies by Gratia (207, 208) established important differences between colicins and bacteriophages, notably the absence of a

bacteriophage-like multiplication of colicins. Later investigations reestablished certain connections; Fredericq (157, 158) showed that particular bacteriophages and colicins attached to the same receptor and discussed other parallels between colicins and bacteriophages (160, 162).

The bacteriophage model also extends to the control of colicin synthesis. Shortly after Lwoff et al. (383) showed that lysogenic bacteria could be induced by ultraviolet (UV) irradiation, it was found that colicinogenic strains could be induced to synthesize colicin by treatment with inhibitors of deoxyribonucleic acid (DNA) synthesis (303, 382). Furthermore, the number of lacunae (clearings, superficially resembling bacteriophage plaques, which are produced by the colicin released from individual colicinogenic bacteria) increases after UV treatment (456). More recently, the inhibition of spontaneous and induced colicin synthesis in *recA*$^-$ strains (27, 251) and the increased colicin titers produced in *tif-1* mutants (Oliver and Hardy, unpublished data) have further strengthened the parallel between the regulatory mechanisms of Col factors and prophages.

Possible relationships between the bactericidal effects of exposure to bacteriophage "ghosts" and to certain colicins, such as E1-K30 and K-235, which affect the energized state of the cytoplasmic membrane, have been pointed out by several authors (132, 162, 377, 431, 447). The immunity conferred by bacteriophage genomes towards the actions of bacteriophage ghosts resembles the immunity determined by Col factors towards specific colicins (447, 574). However, the extent to which colicins provide an appropriate model for the actions of bacteriophage ghosts must await further studies in view of the apparently more drastic effects of the latter agents on the cytoplasmic membrane.

At the moment, any parallels between colicins and bacteriophages remain at the level of analogy, as opposed to homology resulting from their evolution from a common ancestor. No direct evidence for evolutionary relationships between colicins and bacteriophages exists; it remains to be seen whether research at the molecular level may provide evidence of such a relationship. A bacteriophage would seem the most likely colicin ancestor in view of the action of bacteriophage ghosts, the specific immunity towards this action determined by bacteriophage genomes, and the similar effects of UV on lysogenic and colicinogenic bacteria. However, there is no shortage of possible candidates; evolutionary relationships may well exist between such apparently diverse agents as bacteriophage proteins, sex pili, competence factors, bacteriocins, and exotoxins.

Whatever the ancestors of Col factors and colicins, their evolution presupposes the selection of clones which determine colicin-like proteins. The selection pressures responsible for the evolution and for the subsequent maintenance of such clones seem obvious enough. Darwin (110) proposed that, "As species of the same genus have usually, though by no means invariably, some similarity in habits and constitution, and always in structure, the struggle will generally be more severe between species of the same genus, when they come into competition with each other, than between species of distinct genera."

Colicinogeny appears to be a result of this selection pressure among the enterobacteria. However, precisely *what* is being selected—the "unit of selection" (373)—is less clear. Is the most important element subject to selection, the Col factor, segments of the Col factor, the structural gene for the colicin protein, *col*, or the colicinogenic clone of cells? If colicinogeny increases the chances of survival of a clone of cells, then why is the occasional clone in which the *col* gene has become integrated into the chromosome not selected? Such clones almost certainly arise spontaneously in nature, but there are no reports of colicins being determined by chromosomal genes outside the laboratory. Gram-positive bacteria produce bacteriocins, which also are plasmid determined (292, 301, 583). From the host's point of view, an integrated *col* gene may reduce the chance that it will be lost and the cell could also dispense with several plasmid genes, such as those for sex pili.

Selection pressures similar to those responsible for the evolution of colicinogeny have probably been responsible for the evolution of killer strains in eukaryotes, for example, the killer strains of yeast (602) and the *Paramecium* strains which harbor kappa particles (471). In fact, the kappa particles may have a more direct relationship with colicinogenic strains since they appear to be derived from gram-negative bacteria harboring defective bacteriophage genomes which may be induced by UV (471).

A further difficulty in discussing the evolution of Col factors and colicins is that, although the selective advantage of colicinogeny seems obvious, there are very few convincing demonstrations that colicins are effective in the usual environment of *E. coli*, i.e., the gut. There are indications, for example, that colicins are inactivated by intestinal contents, presumably by proteases (325), and three separate investigations using gnotobiotic animals have failed to demonstrate any inhibition, due to colicin, of an *E. coli* strain by a colicinogenic strain in the

intestine. (The role of several bacteriocins produced by gram-positive bacteria is even more uncertain.) Notwithstanding the results of investigations with germfree animals, other studies indicate that colicins modify the intestinal flora, and colicins seem so perfectly adapted for killing *E. coli* in the laboratory that an entirely different role of colicins in nature is improbable.

This review examines the major aspects of bacteriocinogeny from an evolutionary point of view; emphasis is placed on the adaptation of Col factors and colicinogenic bacteria to their environments and on the relationships between Col factors. Both the molecular and the ecological features of bacteriocinogeny are included within such an evolutionary framework. Also, the network of analogies between bacteriocins, bacteriophages, and related entities has been reexamined in the light of recent studies.

The colicins considered here are bactericidal proteins (molecular weight 50,000 to 90,000) which are classified into groups, such as A, B, E, and I, on the basis of their action on bacterial mutants which are resistant to particular groups of colicins. All of the well investigated colicins are plasmid determined and almost all the colicins commonly used were originally investigated and classified by Fredericq (156).

"Colicins" consisting of particles resembling bacteriophage tails also act only on bacteria which are related to the producing strain, but they are clearly distinct from the plasmid-determined colicin proteins. Other bacteriocins, such as those produced by gram-positive bacteria, are considered largely in relation to analogies provided by the well studied colicins and Col factors.

TWO MAJOR GROUPS OF COLICIN FACTORS

Relationships at the Level of Plasmid DNA

Relationships between plasmid DNA molecules can be estimated in various ways to provide information about their origins and their divergence from common ancestors. The nature of the similarities (whether, for example, base-sequence homology between two plasmid DNA molecules is restricted to a particular region or is interspersed with nonhomologous segments throughout the length of the molecule) may provide evidence about the molecular events underlying their divergence. However, studies of the molecular similarities between plasmids relate to only a part of the problem of plasmid evolution and may not provide data about the selection pressures responsible for the evolution of the adaptive features of Col factors and other plasmids.

There are many examples of colicins being determined by extrachromosomal DNA elements and, although strains in which the *col* gene has become integrated into the chromosome can be isolated in the laboratory (164, 317), there are no reports of bacteriocins being normally determined by chromosomal genes. At least, all of the studies made so far on colicinogenic strains isolated by Fredericq from the region around Liége have shown that many of the colicins are plasmid determined.

Genetic analyses made by Fredericq and Betz-Bareau (165, 166) more than 20 years ago first suggested that certain colicins were determined by extrachromosomal DNA. This was first confirmed by nongenetic methods for ColE1-K30 when the DNA was analyzed in the electron microscope by Roth and Helinski (486).

In cell lysates, Col factors, like many other plasmids, are found to be largely in the form of supercoiled closed-circular molecules (245). Whether this is the predominant form in vivo is less certain (243). The physical properties of plasmids and the methods used to study them have been recently reviewed (87, 243, 245, 409).

In considering the relationships between those Col factor DNA molecules for which data are available, the most conspicuous feature is that they fall into two distinct groups with respect to their molecular weights (234, 244; Table 1); the monomer forms of Col factors D-CA23, E1-K30, E1-16, E2-P9, E3-CA38, and K-235 have molecular weights of about 5×10^6, sufficient DNA for about 10 proteins, each of molecular weight 30,000. The monomer forms of Col factors B-K77, Ib-P9, V-K30, and V-K94 have molecular weights at least ten times greater, between 62×10^6 and 94×10^6. This is enough DNA to code for about 100 different proteins, each with a molecular weight of 30,000. The F. ColV. ColB.*trp.cys* plasmid, a recombinant of Col and F factors isolated by Fredericq (164), has a molecular weight of 113×10^6 (256). Many other plasmids, including numerous R factors, seem to fall clearly into one of these two categories (75, 87, 217, 243).

Several genetic and physiological properties are correlated with this fundamental division into two groups; for example, only the larger plasmids are self-transmissible by conjugation, there are fewer copies of the larger Col factors per cell, and the two groups appear to differ in their dependence on host functions required for their maintenance (at least as revealed by their inability to replicate in various *dna* mutants). In addition, the colicins determined by the smaller Col plasmids, E1, E2, E3, and K, are inactive on *tolA* mutants (422, 438), whereas colicins determined by the larger Col plasmids,

TABLE 1. *Molecular weight of colicin factors*

Group I				Group II			
Colicin factor	Mol wt[a]	Method[b]	Reference	Colicin factor	Mol wt[a]	Method[b]	Reference
D-CA23	3.1×10^6	EM	559	B-K77	70×10^6	EM	87
E1-K30	4.8×10^6	EM	486	Ib-P9	61.5×10^6	S	82
E1-16	6.0×10^6	S	234	V-K94	94×10^6	EM	87
E2-P9	6.0×10^6	S	23	VB-K260'[c]	113×10^6	EM	256
E3-CA38	6.0×10^6	S	23				
K-235	6.0×10^6	S	234				

[a] Molecular weights, with electron microscope measurements corrected according to Clowes (87).
[b] Abbreviations: EM, electron microscopy; S, sedimentation velocity centrifugation in neutral sucrose.
[c] ColVB-K260'*trp·cys*, isolated by Fredericq (164).

B, I, and V, are inactive on *tonB* mutants (210). This indicates that there may be two major evolutionary groups of Col factors (234). It will therefore be interesting to see whether this correlation between the molecular weight of the Col plasmids and the action of the colicins they determine holds for other Col factors, particularly in view of the recent finding by Davies and Reeves (114) that 18 different colicins could be classified into two mutually exclusive groups on the basis of their action on resistant (receptorless) or tolerant mutants.

For those Col factors which have been studied most extensively so far, the larger and smaller groups of plasmids have a sufficient number of properties in common to make it convenient to refer to Col factors with molecular weights of about 5×10^6 as group I and to the larger Col factors as group II (234).

Such a division into two groups might have arisen through the divergence of each group from a distinct ancestor, or from convergence of plasmids of diverse origins to give, for example, the group I Col factors. In the latter case, the similarity in size among group I Col factors may indicate simply some minimum number of genes required for replication and segregation to which each has become reduced. Larger plasmids with deletions in one of the genes required for transfer might be the parents of a phylogenetically unrelated group of plasmids if the loss of one gene necessary for transfer made several other genes redundant and which were subsequently lost.

Estimates of the molecular relationships between Col factors which might distinguish between such alternatives can be made by several methods. These include DNA-DNA hybridization followed by separation of double-stranded duplexes on hydroxyapatite columns (216), the examination of DNA heteroduplexes in the electron microscope (286, 469, 513), hybridization followed by treatment with the single-strand-specific endonuclease, S1, derived from *Aspergillus oryzae* (18, 102), or the comparison of DNA fragments produced by restriction endonucleases (291, 556). Not all methods provide the same type of information about the similarity between two plasmids and, particularly, about whether base-sequence homology is restricted to certain regions of plasmids.

The examination of heteroduplexes with the electron microscope probably provides the most complete information in this respect. The use of restriction endonucleases, *Eco*R1, for example, gives comparisons based on the presence of particular nucleotide sequences in plasmids.

Using the heteroduplex technique, Inselburg (286) showed that about 80% of the DNA of group I Col factors E2-P9 and E3-CA38 had base-sequence homologies (demonstration of base-sequence homology in the electron microscope does not necessarily mean that the two sequences are exactly complementary); the heterologous regions were restricted to one section of the plasmids. Ten percent of the DNA of the plasmids appeared heterologous when mixtures of ColE2-P9 and ColE3-CA38 were renatured in 40% formamide; a further 10% was presumably partially homologous since it appeared nonhomologous only when renatured in 70% formamide. Despite the base-sequence homology shown in heteroduplexes, ColE2-P9 has two *Eco*R1-sensitive sites, whereas ColE3-CA38 has three such sites (291). ColE1-K30 has only one *Eco*R1-sensitive site (287, 376, 564). The relationships between ColE2-P9 and ColE3-CA38 are particularly interesting because colicins E2 and E3 have partially similar amino acid sequences (252).

There are no reports about the nature of the heteroduplexes formed between other members of group I, but ColE1-K30 has base-sequence homologies with two plasmids which do not determine colicins. Porter et al. (469) showed that about 70% of the DNA of a plasmid (molec-

ular weight 3.5×10^6) found in *Shigella dysenteriae* Y6R had base-sequence homology with ColE1-K30, which was originally found in *E. coli* K30. Again, the regions lacking homology were confined largely, though not exclusively, to one segment of the plasmids. The plasmid from *Shigella dysenteriae* did not determine the synthesis of a colicin, but it did confer immunity against colicin E1-K30 (no immunity was conferred against colicin E2-P9), suggesting that it might be a defective Col factor. The small plasmid (molecular weight 1.5×10^6) found in *E. coli* 15 also has considerable homology with ColE1-K30 (198), although it determines neither a colicin nor colicin immunity. Not all plasmids with molecular weights of about 5×10^6 can be shown to be related, however. For example, Guerry et al. (217) found that the DNA of two R factors of this size were not homologous with each other.

Sharp et al. (513) showed that about 44% of the F factor had a high base-sequence homology with ColV-K94 and with several F-like R factors. The same region of the F factor was responsible for the homology to both the R factors and to ColV-K94. In agreement with the results of genetic analyses, several cistrons concerned with transfer ability, *tra,* as determined by Ohtsubo et al. (445), are located in the region of homology between F and ColV-K94. Kahn (319) has isolated a mutant of ColV-K94 with a deletion of 11 μm (molecular weight 2.3×10^7) which has lost the ability to determine colicin V synthesis and which has a molecular weight almost exactly the same as that of the F factor.

Numerous studies (144, 216, 407, 513) indicate a basic phylogenetic division between F-like and I-like plasmids (see Relationships Between the Fertility Systems of Col Factors). For example, Guerry and Falkow (216) showed that ColIb-P9 had little base-sequence homology with F-like plasmids when examined by DNA-DNA hybridization followed by hydroxyapatite separation of heteroduplex fragments. It seems likely that all F-like Col factors (ColB-K77, ColB-K98, ColB-K166, ColVB-K260, ColV-K30, ColV-K94) have little homology with the ColI factors.

Although the demonstration of considerable base-sequence homology between two plasmids clearly provides evidence about the evolutionary relationships between them, there may nevertheless be indications of evolutionary relationships between genomes despite the absence of detectable base-sequence homology between them, judging from research with bacteriophage genomes. The genomes of bacteriophages ϕX174 and S.13 are about the same size and have the same number of genes, and, excepting one cistron, the genomes of S.13 can complement those of ϕX174 (see 186). The gene products have similar sizes and functions. By these criteria, the two bacteriophages are closely related. But Godson (186) found that only about 5% of each genome had a high degree of base-sequence homology with the other in heteroduplexes; most of the base sequence was partially homologous, with an average mismatch of about 36% and no "loops" of nonhomologous DNA, as is found between ColV-K94 and F. Similarly, other G phages coding for proteins which closely correspond in both number and size to those of ϕX174, nevertheless had little base-sequence homology with ϕX174 (187). Godson (187) concludes that, with these bacteriophages, it is the number, size, and functions of the proteins overall which are maintained during evolution and not the base-sequence homologies. It will be interesting to see whether F-like and I-like plasmids have a similar overall pattern genetic organization despite differences between the sex pili they determine and the absence of detectable base-sequence homology. There are indications (discussed below) that the mechanisms which control donating ability may be similar in F-like and I-like plasmids.

Changes in plasmid DNA molecules may be expected to come about through mutation and recombination; little is known about the pressures which will select particular changes. Davidson and others (116, 281, 330, 526) have suggested that bacteriophages which can exchange genetic material, such as λ and its close relatives, may evolve genomes consisting of segments which have an all-or-none homology relationship with segments of related genomes. Nonhomology might be selected in certain regions of the chromosome to prevent recombination events from separating genes or other segments of DNA which function coordinately, such as the operator and the gene coding for the repressor. Bacteriophages which could not undergo genetic recombination would exhibit an overall partial homology as they diverged. It is not known how far this suggestion may be applied as a general rule to other genomes, but the heteroduplexes formed between ColV-K94 and F, which readily recombine, have a similar pattern of homologous and heterologous segments.

Recombination between plasmids which have similar insertion sequences (278; and see below) and the recA-independent translocation of plas-

mid DNA segments (352) are probably important factors in producing the pattern of homology seen between F-like plasmids.

Recombination Between Col Factors and Other Plasmids

Recombination between F-like Col factors and other plasmids occurs very frequently under suitable conditions as is well illustrated by the work of Fredericq (164, 170, 171, 172) who examined recombination between the F-like Col factor, ColVB-K260, and various other plasmids.

Fredericq (164) first isolated a stable derivative of ColVB-K260 which carried the chromosomal *trp*, *cysB*, and *tonB* alleles; the markers formed a circular linkage map. The frequency of transduction by bacteriophage P1 of *col* genes from this strain was greatly increased when the recipient carried an F-like plasmid. When the recipient harbored F'*lac*, for example, the percentage of *trp*$^+$ transductants which were also ColB$^+$ increased from 0.1 to 40% and almost all ColB$^+$ transductants could act as donors. Some of these *trp*$^+$ ColB$^+$ transductants could transfer ColB as an independent plasmid, but usually *colB* was found to have recombined with the plasmid already in the recipient. Various lengths of the ColVB-K260 plasmid were found to have recombined with F'*lac*; different combinations of ColV, ColB, *trp*, and *lac* were found on various plasmids. An even greater transduction frequency was found when the recipients harbored ColV-K30 or ColV-K94, but there was no increase in the frequency of *colB* transduction when the recipients harbored ColE1-K30, ColE2-P9, ColIb-P9, or ColIb-P9'*trp*.

When the transductional recipient harbored both ColV-K94 and an R factor, recombinant plasmids with markers from ColB-K260'*trp* and from the R factor could be isolated; recombination between ColB-K260'*trp* and the R factor could be detected only when ColV-K94 was also present (170). Even more complex plasmids were isolated, for example, by transducing a recombinant Col-R factor into a recipient harboring F'*lac*; plasmids with *lac*, *col*, and drug resistance determinants were isolated. The linkage of markers was confirmed by P1 transduction.

Although various factors such as surface exclusion, incompatibility, and restriction act as isolating mechanisms and militate against the formation of recombinants in nature, these results illustrate the remarkable capacity for genetic exchanges among F-like Col factors and other plasmids. Recombination of genes with a resident plasmid may insure the survival of the Col factor if it is unstable by itself in a particular bacterial host.

Many of the recombination events between F-like plasmids may involve insertion sequences. The F factor and several F-like R factors are known to include several of these short nucleotide sequences, such as IS1 (800 base pairs), IS2 (1,400 base pairs) and IS3 (1,400 base pairs) (278). Examination of single strands of ColV-K94 DNA in the electron microscope indicates that this plasmid has three pairs of DNA segments which are in reverse sequence such that they anneal with each other to form a double-stranded "stalk" and a single-stranded "loop" of DNA (513; and Fig. 1); the repeated segments of DNA may be inverted insertion sequences. Inverted sequences which are repeated in the plasmid DNA (palindromes) may be involved in the translocation of DNA segments between plasmids through recA-independent recombination events (see, for example, 352).

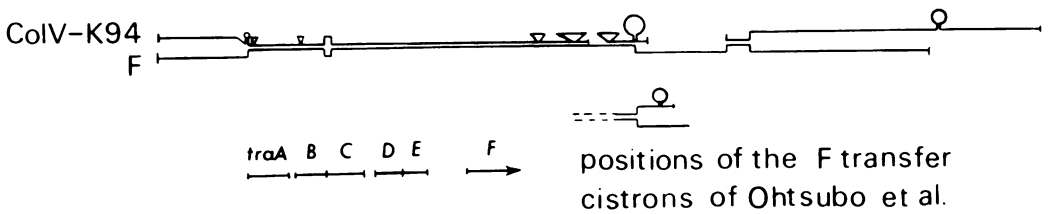

FIG. 1. *Heteroduplex formed between the DNA molecules of ColV-K94 and F (from Sharp et al. [513]). The heteroduplex is shown in a linear form and not as a circular molecule as seen in the electron microscope. Regions of base-sequence homology are shown by the closer parallel lines. The three circular loops on ColV-K94 indicate regions of inverted duplications in this strand; the duplicated segment forms a stalk of double-stranded ColV-K94 DNA. Two sizes were observed (as shown) for the central loop in different ColV-K94 strands. Loops in ColV-K94 DNA due to a deletion or insertion are shown by triangles; the size of the deletion or insertion is indicated by the side of the triangle parallel to the homologous DNA. Gaps in the ColV-K94 strand indicate deletions of F DNA. The F transfer, tra, cistrons of Ohtsubo et al. (445) are located within the regions indicated on the map.*

The origins of the different "species" of plasmids, basically F-like and I-like among the self-transmissible Col factors, remain a mystery; plasmids of each group are often present in the same host and they have little base-sequence homology. The simplest model for the evolution of new species in general, through adaptation to different environments followed by divergence, may be applied to the evolution of the two species of Col factor if separation into different hosts takes the place of geographical separation in the formation of animal species. On being present again in the same host, the plasmids may already be sufficiently dissimilar to prevent much recombination between them. Alternatively, if large segments of the plasmids are important units of selection, recombinants may be less well adapted so there would be a further selection for specific isolating mechanisms to prevent recombination.

SURVIVAL OF COL FACTORS IN BACTERIAL CELLS: ADAPTATION TO THE HOST CELL ENVIRONMENT

Host Range

Colicin factors are adapted to their immediate environment, the host cell, so that they are efficiently maintained and replicated. The corollary is that Col factors and other plasmids have a limited host range (111, 407, 409).

F-like plasmids are generally less stable (i.e., more likely to be lost) in *Salmonella typhimurium* than in *E. coli* or *Proteus mirabilis*, whereas many I-like plasmids are unstable in *P. mirabilis* but stable in *S. typhimurium* (111, 586). Group I Col factors also appear to differ in their stability in different hosts; ColE plasmids, but not ColK plasmids, are stable in *S. typhimurium* LT2 (457), but duplication of ColE1-K30 is abnormal in *P. mirabilis* (194, 196).

Data are not available for all Col factors, but the stability of F-like and I-like plasmids in various hosts is correlated to some extent with the frequency with which they are found in various bacteria (407). About 77% of plasmids isolated from *E. coli* strains are fi^+, which is closely correlated with the F-like character, compared with about 33% in *S. typhimurium* strains.

Experimental Approaches to the Problem

The replicon model provides the most useful framework for considering the maintenance and replication of Col factors (302). This focuses attention on two important aspects of plasmid maintenance: firstly, the regulation of the number of copies per cell through regulation of the initiation of plasmid replication and, secondly, the postulated attachment of plasmids to the cytoplasmic membrane to permit their orderly separation into daughter cells. Consideration of both of these points illustrates differences in the adaptation of Col factors from groups I and II to the bacterial cell.

The survival of Col factors in bacterial cells implies that their replication is coupled to the growth rate of the host. The model proposed by Pritchard et al. (472, 473, 474) is concerned with this aspect of the process – the nature of the biological clock in the system – although little is known about the biochemistry of the mechanism.

The most conspicuous difference between the survival of group I and group II Col factors in bacteria is the number of copies of the plasmids per cell, presumably a consequence of the mechanism controlling the initiation of Col factor replication. Experiments which provide information about the molecular basis of such differences in the adaptation of Col factors to the host-cell environment may be divided into three groups. (i) Specific inhibitors may be used to show the classes of macromolecules which are required at different stages in the replication cycle. (ii) Col factors may be transferred to mutants, especially temperature-sensitive (*ts*) mutants, to determine which functions required for maintenance are provided by the host cell. Mutant Col factors which are unable to replicate or be maintained in wild-type hosts may also be isolated. (iii) Intermediates of Col factor replication in vivo or in vitro may be examined.

Regulation of the Number of Col Factors per Cell

There are between 5 and 25 copies of Col factors E1-K30, E2-P9, E3-CA38, and K-235 per chromosome-equivalent under most growth conditions (234, 244). Several R factors and other plasmids with molecular weights of about 5×10^6 are similarly present in multiple copies per cell (87, 536).

Although data are not available for all members in group II, it seems likely that they resemble F and several self-transmissible R factors which replicate so that there are no more than one or two copies per chromosome-equivalent (87). Values reported for ColIb-P9 (82, 296), F.ColV.ColB.*trp.cys* (256), ColB-K77 (87), and ColV-K94 (87) are consistent with only one or two plasmids per chromosome-equivalent.

It is believed that F particles replicate at a particular point in the cell cycle, judging from the increase in β-galactosidase activity after induction of F'lac^+ cells in a synchronously growing culture (604). This does not appear to correspond to a particular point in the chromosome

replication cycle or the cell division cycle; it may correspond to a critical cell mass (474).

None of the group II Col factors which have been shown by physical techniques to exist in only one or two copies per chromosome have been examined to determine whether they initiate duplication at a particular point in the cell cycle. In fact, the F-like plasmid, ColB-K260'*trp.lac* isolated by Fredericq (164), does not appear to replicate at a special point in the cell cycle (605). The expression of the *lacZ* gene after induction was used to measure the relative number of plasmid copies during synchronous growth. β-Galactosidase activity increased almost exponentially; there was little evidence of a step-wise increase which might indicate that replication of all the plasmids in the cells was initiated at a particular point in the cell cycle. The results indicated that copies of the plasmid were selected at random from a pool throughout the cell cycle. The number of plasmid copies per chromosome was not measured directly.

Density-shift experiments by Bazaral and Helinski (24) demonstrated that some copies of ColE1-K30 duplicate twice before the chromosome completes a round of duplication. While the chromosome duplicated twice, about 25% of ColE1 plasmids duplicated twice, about 50% duplicated once, and about 25% did not duplicate at all. The most straightforward interpretation is that plasmids were selected at random for duplication, irrespective of when they had completed previous rounds. Similar results have since been obtained for a small R factor in *Proteus mirabilis* (487, 555) and the small plasmid in *E. coli* 15 (199).

The "relaxed" mode of ColE1-K30 replication is inhibited when it is combined with plasmid pSC101 (a nonconjugative plasmid [molecular weight 5.8×10^6] obtained by shearing the self-transmissible R-factor R6-5) to form a single hybrid plasmid (559). Plasmid pSC101 replicates under "stringent" control such that there are only one or two copies per chromosome equivalent. The hybrid ColE1-pSC101 plasmid also replicates under stringent control. The stringent character of pSC101 is dominant in the *cis* configuration but not in the *trans* configuration when the two plasmids are present separately in the same cell (559).

Functions Contributed by Hosts and by Col Factors

The inhibitors chloramphenicol and rifampin may be used to determine whether protein or ribonucleic acid (RNA) synthesis is required for particular aspects of plasmid replication. Protein synthesis is required before rounds of replication of the chromosome or of the plasmids ColIb-P9, ColV-K94, or F can be initiated; at most only one round of replication is initiated after removal of required amino acids or after the addition of chloramphenicol (24, 192, 340, 538). It is possible, therefore, that the larger plasmids have become adapted to their hosts through their ability to respond to proteins which initiate chromosome replication. However, replication of F is not initiated at the same time as the initiation of chromosome replication (474).

Whatever the nature of these proteins (and irrespective of whether they are determined by the plasmid or the chromosome), initiation of ColE1-K30 replication is not dependent upon their continued synthesis. Indeed, in a growth medium of acid-hydrolyzed casein and glucose, the rate of ColE1-K30 duplication in the presence of chloramphenicol increases to about eight times the rate found in cultures without chloramphenicol (77). ColE1-K30 continues to replicate for at least 15 h in the presence of chloramphenicol. This makes ColE1-K30 especially useful for cloning genes which are introduced into the plasmid, particularly since it has a single *Eco*R1-sensitive site (253, 552, 559).

Hershfield et al. (253) introduced DNA which included the *trp* operon into ColE1. Levels of this composite plasmid increased after chloramphenicol treatment to 45% of the total cell DNA. In the absence of chloramphenicol, the level of *trp* messenger RNA (mRNA) and *trp* enzymes corresponded to those expected on the basis of the number of ColE1' *trp* plasmids per cell.

Replication of the *E. coli* chromosome and of several bacteriophage genomes and plasmids also requires RNA synthesis; the RNA is believed to serve as a primer for the extension of DNA chains (63, 353, 499). Studies with rifampin, an inhibitor of RNA polymerase, first suggested a role for RNA in ColE1-K30 replication. Although replication continues in the presence of chloramphenicol, it ceases when rifampin is added, either alone or with chloramphenicol (78, 79).

In fact, replication in the presence of chloramphenicol causes an abnormal retention of RNA in one of the strands of the ColE1-K30 duplex (36, 248); closed-circular molecules are nicked by alkali or ribonuclease (RNase) H (which acts only on the RNA component of DNA-RNA hybrids [36, 368]) and converted to the open-circular form. Williams et al. (596) have estimated the number of nucleotides and the base composition of the RNA fragments.

Two methods (the amount of radioactivity released from labeled ColE1 by RNase H and pancreatic RNase A, and secondly, the number of ^{32}P-labeled nucleoside triphosphate molecules incorporated into ColE1 previously treated with RNase) both gave estimates of 25 or 26 ribonucleotides per molecule.

Direct confirmation of the role of RNA in ColE1 replication has been provided by Sakakibara and Tomizawa (493), who showed that about 20 ribonucleotides were covalently linked to 6S DNA molecules (presumably "Okazaki pieces") which were intermediates in the replication of ColE1-K30 in cell extracts. Oka and Inselburg (446) also found that RNA was attached to newly synthesized DNA molecules (5-S13S) during ColE1-K30 replication in minicells.

Information about the products contributed by hosts and Col factors may be obtained from studies on the ability of Col plasmids to survive in mutant bacteria. Mutations affecting chromosome replication are generally divided into two categories: those which affect the initiation of replication and those which affect chain elongation. It is sometimes difficult to draw conclusions about the requirements for plasmid replication because conditional mutants may retain a little enzyme activity and, in addition, mutations may affect one activity of a complex while leaving other activities intact.

Four genes, *dnaA*, *dnaC*, *dnaH*, and *dnaI*, are known to be essential for the initiation of chromosome replication (see 214, 499). The precise nature and function of the *dnaA* gene product is unknown, but many F-like plasmids, including ColB and ColV factors, seem to determine an equivalent product; ColB-K77, ColB-K98, ColB-K166, ColV-K30, and ColV-K94 can suppress the *ts dnaA* mutation at the restrictive temperature through "integrative suppression" (419, 425, 426).

There are conflicting reports concerning the dependency of ColE1-K30 replication on the *dnaA* gene product. Goebel (191, 192) found that the synthesis of ColE1-K30 DNA decreased much more rapidly at the nonpermissive temperature in a *ts dnaA46* mutant than did the synthesis of chromosomal DNA or that of the F-like plasmids R1*drd*19 or HlyPM 152. These two plasmids could initiate a few further rounds of duplication at the restrictive temperature, but no further rounds began after the completion of chromosomal replication. However, Collins et al. (90) found that ColE1-K30 replication continued at an almost undiminished rate in *dnaA46* and *dnaA83* mutants at the restrictive temperature despite the expected decrease in chromosomal DNA synthesis upon completion of rounds of replication. However, ColE1-K30 replication is dependent upon the *dnaC* gene product (90).

Kingsbury and Helinski (332) demonstrated that ColE1-K30 could not be maintained in the *polA1* mutant which has a very low level of DNA polymerase I activity (123), but which retains almost normal levels of the 5′ → 3′ exonuclease activity associated with this enzyme (349, 367). Other small plasmids are similarly unable to survive in the *polA1* mutant; these include the small plasmid found in *E. coli* 15, ColE1-16, ColK-235, CloDF13, and to some extent ColE2-P9 (197, 332, 334, 335, 578; K. G. Hardy, unpublished data).

Chromosome replication and the replication of Col factors Ib-P9 and V-K30 continued in the *polA1* mutant (332), although it is known that the gaps between newly synthesized fragments of chromosomal DNA are joined more slowly (359, 449).

Whether the different behavior of group I Col factors in strains with very low levels of polymerase I activity is due to a fundamentally different mode of discontinuous DNA synthesis remains unclear. In the synthesis of chromosomal DNA, it is believed that the 5′ → 3′ exonuclease activity of DNA polymerase I removes the RNA primers required for the synthesis of short DNA fragments by DNA polymerase III. The polymerase activity of the enzyme then joins the discontinuous DNA strands, Okazaki fragments, synthesized by DNA polymerase III (349, 353, 450, 499). It is possible, therefore, that there is sufficient polymerase I activity remaining in several *polA* mutants to enable them to join Okazaki fragments, albeit less efficiently, in chromosome replication and in the replication of larger plasmids, but that the smaller plasmids have a more stringent requirement for normal levels of the polymerase.

Although synthesis of ColE1-K30 DNA stops immediately at the restrictive temperature in *ts polA* mutants, almost all copies retain the closed-circular form; they are simply diluted out during subsequent cell divisions (137, 334). On returning to 30 C after 1 h at the restrictive temperature, [^{3}H]thymidine was immediately incorporated into ColE1-K30 DNA.

Whether there are differences between the dependencies of larger and smaller Col factors on DNA polymerase III, as indicated by their ability to replicate in *ts dnaE* mutants, also remains unclear. DNA polymerase III is essential for chromosome replication (182, 440). Goebel (190) found that ColV-K30 and ColIb-P9 replicated at the permissive temperature in a *ts dnaE* mutant (*E. coli* 1026), but no counts were incorporated into plasmid DNA after incubation

for 1 h at 45 C.

In contrast, counts were incorporated into ColE1-K30 DNA, which replicated semi-conservatively at the restrictive temperature and was found to be largely in the closed-circular form on isolation (190). The interpretation of the experiment is complicated by the fact that no ColV-K30 DNA was synthesized at 45 C in the *dnaE*$^+$ host, whereas ColE1-K30 can replicate at this temperature (195); and the mutant may also be leaky (550). Collins et al. (90) found that ColE1-K30 DNA synthesis was considerably reduced in a *ts dnaE486* mutant at the restrictive temperature.

Notwithstanding certain differences in their behavior in mutant bacteria, it would therefore be premature to conclude that group I and group II Col factors depend on different polymerases for their replication, particularly in view of the roles for both DNA polymerase I and III in the replication of bacteriophage genomes and the *E. coli* chromosome (353, 499, 550).

In a different approach to the contributions of the host to Col factor survival, Kingsbury et al. (333, 334, 335) selected temperature-sensitive bacterial mutants on the basis of their inability to maintain ColE1-K30 at 42 C; many of these mutants were found to be unable to maintain other plasmids besides ColE1. The mutants could be divided into three groups: class I mutants could maintain none of the seven plasmids tested (ColE1-K30, ColE2-P9, F'*lac*, ColV-K94, R1*drd*19, ColIb-P9, R64); class II mutants could maintain only I-like plasmids; class III mutants, some of which were shown to possess a *ts* DNA polymerase I, could maintain all the plasmids except ColE1-K30.

Temperature-sensitive mutants of ColE1-K30, which were not maintained at 42 C, were also isolated, indicating that this plasmid determines at least one protein essential for its survival in host cells. None of the plasmids examined could complement the chromosomal mutations to enable ColE1 to be maintained, but some of the *ts* mutants of ColE1-K30 could be maintained at the restrictive temperature if F'*lac* was also present in the cells.

ColIb-P9 confers resistance to UV and to other agents which damage DNA (272). Numerous I-like R factors also confer protection against UV (131, 392, 521) and increase the mutation rate after irradiation (273, 385, 386). Many of these R factors also determine colicin I synthesis. Whether this protection is due to a product which is also involved in Col factor duplication and maintenance is unknown, but it is not essential for ColIb survival in cells; mutants have been isolated which do not increase UV resistance or the mutation rate (274). MacPhee (387) has shown that R-Utrecht, which confers protection against UV, determines a DNA polymerase activity which may be involved in closing single-strand gaps in DNA.

Intermediates

The restriction endonuclease, *Eco*R1, produces a double-strand break at one specific site in the ColE1-K30 molecule. (This site may be in the structural gene for colicin E1, since the insertion of DNA at this point leaves the plasmid unable to determine colicin E1 synthesis although immunity against colicin E1 is unaffected [253]). The origin of ColE1-K30 replication has been determined in relation to this site using molecules isolated from bacterial cells, minicells or a cell-free system (287, 376, 564). Measurements of the length of the two unreplicated segments of ColE1 molecules in various stages of the replication cycle indicated that replication proceeded in one direction from an origin 17 to 18% of the total length of the plasmid from the *Eco*R1-sensitive site (Fig. 2). The round of replication terminates at a point close to the origin (376, 494). (Unidirectional replication of ColE1-K30 is in marked contrast to the bidirectional replication of the *E. coli* chromosome [34, 68, 393]).

Catenates comprising two interlocked rings of ColE1-K30 molecules are sometimes found in cell lysates, including preparations from *recA*, *recB*, and *recC* strains (196), and these may be an intermediate of some kind (189, 195, 285, 290). Catenates accumulate at 49 C in *E. coli* and, on returning to 30 C, are converted into monomers (195). Catenates composed of an open-circular molecule and a closed-circular molecule were also detected when ColE1-K30 replicated in cell extracts (493).

A protein found associated with ColE1-K30 which almost certainly has an important role in replication is the "relaxation protein" found by Clewell and Helinski (80). Most of the supercoiled closed circles in lysates of ColE1$^+$ bacteria can be converted, or relaxed, to the open-circular form by agents which destroy or denature proteins. Closed-circular DNA molecules which can be so converted are called relaxation complexes (80). Other plasmids can be relaxed in a similar manner, including ColE2-P9 (36, 81), ColE3-CA38 (81), ColIb-P9 (82), F (341), bacteriophage ϕ29 (452), the small plasmid from *E. coli* 15 (397), and the R factors R6K (375) and ΔT (279).

Relaxation complexes formed by different plasmids can be distinguished, for example, by their degree of resistance to relaxation either by Pronase or by incubation at high tempera-

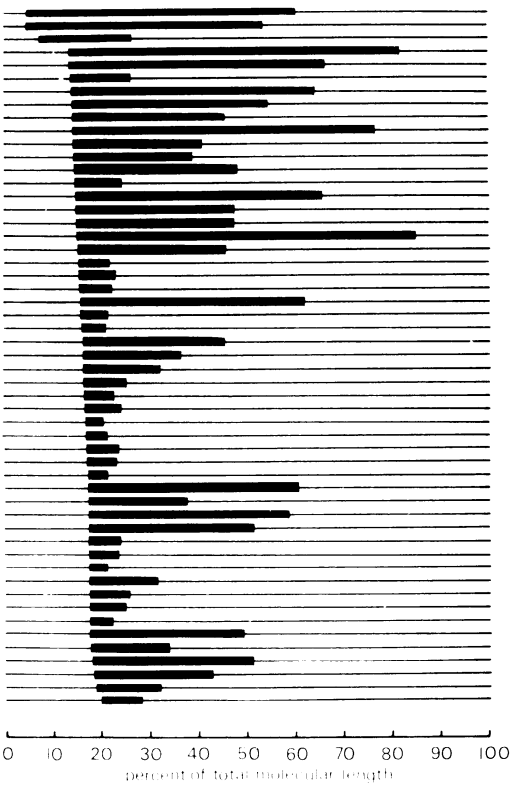

FIG. 2. *Determination of the origin of ColE1-K30 replication in minicells (from Inselburg [287]). ColE1-K30 molecules isolated from minicells were treated with the restriction endonuclease, EcoR1, to produce linear molecules which were examined in the electron microscope. The lengths of the replicated and unreplicated segments in each replicating molecule were measured. In the figure, the molecules are aligned with the shorter unreplicated segment to the left. Symbols: ■, the segment between the two replication forks; ——, the unreplicated segments. These results, and similar data obtained by Lovett et al. (376) (using bacterial cells) and Tomizawa et al. (564) (using cell-free extracts), indicate that ColE1-K30 replication proceeds in one direction from an origin about 18% of the total length of the molecule from the EcoR1 cleavage site.*

ture (36, 83, 341), but the nick introduced on relaxing the complexes of ColE1-K30, ColE2-P9, or F in each case occurs always in only one of the two polynucleotide strands (36, 80, 341). This was shown by sedimentation in alkaline sucrose gradients to separate linear and circular strands of the relaxed plasmids. On centrifuging the two strands to equilibrium in CsCl containing an excess of poly(UG), which preferentially binds to the DNA strand with the lower GC content, the nicked strand was found to be the heavier of the two.

A role for the relaxation protein in ColE1-K30 replication is indicated by the finding that the single-strand break which is produced by relaxation maps at the same position as the origin/terminus of replication (375). Upon relaxation with sodium dodecyl sulfate, two protein components of the relaxation complex (with molecular weights of 11,000 and 16,000) are lost while the protein which remains (molecular weight 60,000) is found to be attached to the 5′ end of the nicked strand (Guiney and Helinski, cited in 376). The removal of the smaller proteins may therefore activate an endonuclease which is specific for one of the two strands in the DNA duplex. The protein found associated with the small plasmid in *E. coli* 15 appears to be an endonuclease which requires divalent cations for its action (398, 399).

However, hypotheses implicating the relaxation protein in replication must take account of the finding that replication continues at an increased rate in the presence of chloramphenicol. Clewell and Helinski (84) have suggested that the relaxation protein may dissociate and reassociate throughout the period of chloramphenicol treatment. Furthermore, closed-circular ColE1-K30 molecules completed a round of replication in cell extracts derived from a Col^- strain even though the closed-circular molecules added to the extracts were insensitive to relaxation (565). In vitro replication also took place in the presence of chloramphenicol or puromycin, but not in the presence of rifampin (565).

On the model proposed by Jacob et al. (302), association of replicons with the membrane is essential for their replication and segregation into daughter cells at cell division. It is possible that this may not be necessary for certain group I Col factors which may be present in sufficient numbers to insure that each daughter cell receives at least one copy, but a strictly stochastic inheritance of these Col factors remains to be established. There are indications that the larger and smaller plasmids differ in their association with the membrane. Only 30% of closed-circular ColE2-P9 DNA is membrane bound compared with 70% of closed-circular ColIb-P9 DNA, judging from their co-precipitation with crystals of cadmium lauroyl sarkosinate (130). About 20% of ColE1-K30 was found to be membrane bound; most of the Col factor DNA remained in the supernatant after a clearing spin to sediment the membrane-bound fraction (515). About twice as much labeled ColE1-K30 DNA was deposited when the cells were pulse labeled for 45 s or 90 s, suggesting that ColE1 DNA synthesis took place on the membrane.

These differences between the larger and

smaller plasmids may also be related to the more limited association of smaller plasmids with the folded chromosome of *E. coli*. Kline and Miller (342) found that about 90% of closed-circular F plasmids were associated with the folded chromosome (541, 543), compared with less than 10% of ColE1-K30 molecules.

Incompatibility

Incompatibility is the inability of two plasmids to be maintained in the same cell. Many plasmids have been classified into incompatibility groups; any two members from the same group are unable to survive stably in the same host cell.

Not all Col factors have been classified into these groups since closely related Col factors, such as the various ColB or ColV plasmids, often cannot be easily distinguished, for example, by characteristics of their colicin. Within the F-like group of plasmids there are at least five incompatibility groups. With representatives of each group, these are: FI (F'*lac*), FII (R1), FIII (ColB-K98), FIV (R124), and FV (JR72) (112, 179, 240, 242). Most F-like R factors belong to group FII; groups FIII, FIV, and FV are each represented by only one member. R factors in group FII and the F factor (FI) can coexist with ColB-K98 and ColB-K166 (179). ColV-K94 can apparently coexist with F when this plasmid is integrated to form an Hfr (384).

I-like plasmids can also be classified into incompatibility groups (241, 407). ColIa-CA53 and ColIb-P9 have been placed in group Iα with R factors R144, which also determines colicin I synthesis, and R64 (241). However, not all ColIa factors are incompatible with ColIb factors (276).

A locus for incompatibility, *inc*, has been mapped on the F factor between $\phi_{\parallel}^{R}$ and *ori*, the origin of transfer (592), but the mechanism of incompatibility is unknown. On the replicon model, replication is initiated at a specific site in the cell, probably on the cytoplasmic membrane; incompatible plasmids compete for a limited number of sites. An alternative explanation (473) is based on the properties of a postulated inhibitor of replication which is produced after initiation of a round of plasmid replication and is diluted during subsequent cell growth until a critical level is reached, whereupon a new round is initiated. An incoming plasmid begins to replicate and thereby increases the concentration of inhibitor. Since replication is initiated only when the concentration of inhibitor again falls to a critical level due to an increase in cell volume, the incoming plasmid will segregate before the completion of further rounds of replication. On this model, compatible plasmids are those which recognize different inhibitors.

Incompatibility is also found between pairs of smaller nontransmissible plasmids (289, 536). Inselburg (289) found that *E. coli* strains harboring both ColE2-P9 and ColE3-CA38 segregated singly colicinogenic clones, whereas strains harboring ColE2-P9 and ColE1-K30, or ColE3-CA38 and ColE1-K30, were stable. However, ColE1-K30 was sometimes lost from strains harboring all three ColE plasmids. Inasmuch as other evidence suggests that ColE2-P9 and ColE3-CA38 are more closely related to each other than either is to ColE1-K30, these results suggest that incompatibility also indicates evolutionary relationships among group I Col factors. Furthermore, despite their differing number of copies per cell, at least one aspect of their means of survival in host cells may be common to both group I and group II Col factors.

RELATIONSHIPS BETWEEN THE FERTILITY SYSTEMS OF COL FACTORS

The self-transmissible Col factors studied so far belong to either the F-like or I-like groups of plasmids. This division is based on the nature of the sex pilus and is confirmed by hybridization studies which show more extensive base-sequence homology between members of the same group than between members of different groups (144, 216, 513). Self-transmissible plasmids which are neither F-like nor I-like have been described in strains of enterobacteria (for example, 44, 329), but none has yet been reported to determine a colicin.

Within each of these two main groups of self-transmissible Col factors, several features of the fertility system may be used to define subgroups. Further details of the relationships indicated by fertility inhibition, immunological characteristics of the sex pilus, and surface exclusion are discussed by Meynell et al. (407).

Sex Pili

Studies on the sex pili determined by Col factors indicate that ColB and ColV factors are more closely related to each other than either is to the ColI factors. Serotyping within the F-like or I-like groups demonstrates the affinities between Col factors and R factors and indicates a particularly close relationship, if not identity, between the fertility systems of ColV-K94 and F.

In the electron microscope, F-like pili determined by ColV and ColB plasmids, excepting ColV-CA7 which is apparently a defective (*tra*$^-$) F-like Col factor (93, 384), are up to 20 μm long and many occur as tangled threads adhering to the cell. I-like pili, determined by ColIa

and ColIb plasmids, are no more than 2 μm long (365).

The male-specific phage, μ2, has a high efficiency of plating on F^+, ColV-$K94^+$, and ColV-$K30^+$ bacteria indicating that almost all the cells are producing sex pili (68, 384). F-specific bacteriophages do not form plaques on $ColB^+$ cultures, but the sensitivity of a few bacteria in the population can be shown by adding a known titer of bacteriophage and reestimating the titer after allowing time for bacteriophage multiplication (403, 404).

ColIa and ColIb plasmids and the sex factor I-16 originally found with ColE1-16 confer sensitivity to the I-specific filamentous bacteriophages, If1 and If2 (409, 410).

Electron microscopy of piliated cells which have been treated with anti-pilus antibody has been used to demonstrate evolutionary relationships within the F-like or I-like groups (363, 365). Serotypes within the F-like or I-like groups may also be distinguished by the inhibition of conjugation brought about by specific antisera (229). Four serotypes can be distinquished using cross-absorbed sera raised against F-like pili. The pili determined by F or ColV-K94 could not be distinguished, but ColV-K30 and a derepressed mutant of ColB-K77 determined pili different from those produced by F^+ bacteria (328; E. Meynell, personal communication; Table 2).

I pili determined by various Col factors and R factors could be divided into only two serotypes; one was represented by only a single member, R64, and the other type was represented by ColIb-P9, I-16, R144, R163, and R538I. Both R144 and R163 also determine the synthesis of colicin I (402).

Fertility Inhibition

Fredericq and Betz-Bareau (168) showed that the presence of certain Col factors in F^+ bacteria reduced the ability of the cell to donate F. This is termed "fertility inhibition" (585); plasmids which inhibit the fertility of F^+ cells are termed fi^+.

Within the F-like group of plasmids, fertility inhibition may be used to assess the specificities of the mechanisms regulating the production of sex pili and associated functions required for transfer. Plasmids which do not belong to the F-like group, for example ColE2-P9, may be operationally fi^+, but this is not due to the production of a repressor effective on the operator of F-like plasmids since o^c mutants are equally affected (401).

Genetic studies indicate that fertility inhibition brought about by ColB factors and fi^+ R factors can be explained by a classical negative control system of the operator-repressor type. That is, ColB-K98, for example, determines a repressor which inhibits the synthesis of F-pili as well as the synthesis of ColB pili. Two types of derepressed (*drd*) mutants of F-like R factors were isolated and distinguished in strains harboring the *drd* mutant and ColB-K98 which produces repressor; by analogy with the *lac* operon, i^- *drd* mutants were repressed, whereas o^c *drd* mutants continued to synthesize sex pili constitutively (179). In further confirmation of the repressor-operator hypothesis, mutants of F have been isolated which are insensitive to the repressing effects of ColB-K98 (178). F and ColV-K94, which determine constitutive sex pilus synthesis, therefore appear to be i^- plasmids.

However, studies with ColB factors and several F-like R factors suggest that the fertility control systems of these plasmids have evolved so that they no longer have identical specificities. For example, ColB-K77, unlike ColB-K98 and ColB-K166, does not greatly reduce the frequency of transfer of the F factor. The control systems of ColB-K77 and R124 are very similar, however; *drd* mutants of either plasmid may be repressed by wild-type strains of the other member of the pair (E. Meynell, personal communication).

An additional factor involved in fertility repression has been postulated by Finnegan and Willetts (150) to account for the results of experiments with transient heterozygotes. Mutants of F'*lac* were isolated which were transferred at high frequency from cells simultaneously harboring the fi^+ R factor, R100. On the simplest model, all these should be o^c mutants of F'*lac*. However, when they were examined in "transient heterozygotes" they were shown to be of two different types. Transient heterozygotes were made by introducing the mutant F'*lac* into a strain already harboring F'*his* and R100. The donor of the mutant F'*lac* was then killed with bacteriophage T6 and the heterozy-

TABLE 2. *Serotypes of sex pili*[a]

Serotypes of F-like:			Serotypes of I-like:		
F	R1	R538F	R100	R64	ColIb-P9
F ColV-K94	R1	R538F	R100 R136 R192	R64	R144[b] R163[b] R538I ColIb-P9 I-16

[a] From Lawn and Meynell (363).

[b] These plasmids also determine colicin I synthesis.

gotes containing two F' factors and R100 were mated with an F^- recipient for 30 min. Transfer of the mutant F'*lac* was inhibited. However, when the intermediate strain harbored only the newly introduced F'*lac* and R100, the F'*lac* was transferred almost as frequently as from an F^- R^- intermediate.

The result suggested that the resident F'*his* determined a *trans*-acting product, P_F, which was required to produce an immediate repression by R100. The mutant F'*lac* was assumed to be defective in the production of P_F. Further mutants of F'*lac* were isolated which were insensitive to the combined action of P_F and the fertility repressor, I, determined by R100. These mutants were assumed to be defective in the site of action of the inhibitor (that is, equivalent to o^c mutants) and were designated *traO* (or *finO*) mutants.

In testing the specificity of the postulated P_F products in heterozygotes, Finnegan and Willetts (151, 590) found that the products determined by ColV-K94, but not those determined by ColVB-K260 or ColB-K98, also acted on F.

The results of complementation tests between plasmid genes required for transfer, *tra* cistrons (444, 445, 590), are in agreement with data obtained by Sharp et al. (513) on the base-sequence homology in heteroduplexes formed between ColV-K94 and F. Using transient heterozygotes, 12 cistrons necessary for transfer have been determined (2, 294, 591, 593). Apart from their genes for surface exclusion, *traS*, ColV-K94, and ColVB-K260 can complement all the *tra* genes of F (590, 595).

Less is known about the control of donating ability determined by I-like Col factors, but *drd* mutants of ColIb-P9, I-16, and numerous I-like R factors have been isolated (140, 405, 407, 442), suggesting that the mechanism may be similar to that found with the F-like Col factors.

Exclusion

An integral feature of the plasmid-determined transfer system is the surface exclusion shown by exponentially growing strains harboring plasmids; they are poor recipients when mated with donors harboring identical or closely related sex factors (366). The evolution of surface exclusion is presumably essential to the bacterial mating system to prevent a clone of cells from continually infecting each other, or even to prevent self-infection if a cell should produce a sex pilus which then attaches to part of the same cell!

Exclusion occurs among both F-like and I-like plasmids (275, 408, 409, 595) and its specificity indicates evolutionary relationships within the F-like or I-like groups. The specificity of surface exclusion is often closely correlated with the specificity of the sex pilus, even to the serotype level (229) and is directed against the type of pilus which transfers the DNA rather than against the DNA itself. Thus, donors having sex pili composed of two types of pilin subunit, those of an Hfr and those of an F-like R factor, are not excluded by F^+ recipients, presumably because the R factor subunits are unaffected by the F-directed exclusion and are "dominant" in this instance (229). However, ColV-K94 apparently shows mutual exclusion with R538F but not with F (320, 595), despite the similarity of the sex pili determined by F and ColV-K94.

ColV-CA7, on the other hand, shows surface exclusion towards F^+ and Hfr donors, although it is not self-transmissible (93, 384). Quite possibly, transfer-deficient mutants have been selected during the 50 years of laboratory cultivation of this strain since its isolation by Gratia; donating ability may have selective disadvantages under certain conditions (129).

Transfer of Chromosomal Genes

Self-transmissible Col factors also act as sex factors and contribute to the flow of chromosomal genes between bacteria. Presumably this is a factor which has contributed indirectly to the survival of Col factors as sex factors. Bacteria which accommodate these plasmids contribute more genes (including those for Col factor maintenance) to future generations through the transfer of chromosomal DNA to other clones.

F-like Col factors, ColB-CA18, ColB-K77, ColB-K98, ColB-K166, ColV-K30, ColV-K94, and ColV-K260 are able to transfer chromosomal genes from *E. coli* K-12 at about the same rate as that promoted by F (88, 92, 163, 321, 384, 418). High-frequency transfer preparations (537) of the ColB factors are used in order to make valid comparisons between repressed and derepressed systems (418). In all cases, recombination of chromosomal loci occurs at a frequency of about 10^{-4} to 10^{-5} per donor for any particular locus.

Among the I-like plasmids, ColIb-P9, isolated from a strain of *Shigella sonnei*, and the sex factor I-16 from *Salmonella typhimurium* are exceptional in bringing about recombination at a frequency of only about 10^{-7} to 10^{-9} per donor even when derepressed mutants are used. In contrast, R144*drd* and R163*drd*, originally found in strains of *Salmonella*, determine colicins and I-like sex pili which are indistinguishable from those determined by ColIb-P9*drd* (92, 363), yet they induce recombination at about 10% of the frequency shown by F.

The frequency of recombination promoted by F, ColB, and ColV factors is reduced in *recA*$^-$ donors to about 10^{-9} per donor (88, 271, 418). Recombination promoted by ColIb-P9 from both *recA*$^-$ and *recA*$^+$ donors is at the same level, about 10^{-9} per donor. This suggests that recombination events brought about by ColIb-P9 are due to a second, more inefficient process which can be detected with other plasmids only when *recA*$^-$ donors are used.

The frequency of stable Hfr strains in nature is unknown. On the one hand, there would appear to be little selective advantage to plasmids which form Hfr strains since they will only rarely be transferred intact to other strains. However, integration into the chromosome may decrease the probability of loss from the host.

The preferential integration of F and ColV factors at certain sites on the chromosome suggests that homologous regions, probably insertion sequences (491), are necessary for Hfr formation. Several Hfr strains have been derived from ColV$^+$ bacteria (164, 317, 318). Fredericq (164) isolated a variant of ColVB-K260 which had lost the *colV* determinant and which had integrated into the chromosome to form an Hfr with *mtl* as proximal marker and *strA*, closely linked to *colB*, as terminal markers. Most of the Hfr strains isolated by Kahn (317) after treating ColV-K94$^+$ cells with a mutagen also had an origin near *xyl*. Some clones no longer produced colicin V. Some of these strains may not have been Hfr's even though they preferentially transferred from the *xyl* locus at high frequency, since most recipients also received the Col factor. This type of transfer resembles that promoted by the R factor R1*drd*19 (461), and may be related to the donation of chromosomal genes by F′ factors, where a fragment of chromosomal DNA in the F plasmid apparently results in frequent but ephemeral integration into the chromosome at the homologous region (3).

There are no reports of stable Hfr strains formed by ColB*drd* mutants, but ColB plasmids can produce integrative suppression of *dnaA* mutants which transfer chromosomal genes at high frequency (419). ColIb-P9 and the R factor R144*drd*3 are unable to suppress the *dnaA* mutation even though R144*drd*3 can transfer chromosomal genes at frequencies approaching those of F (419).

Transfer of Group I Col Factors

The considerable selective advantages conferred on group I Col factors, whose transfer is promoted by self-transmissible plasmids, has presumably led to the selection of group I plasmids which are transferred at a very high frequency to other bacteria from hosts harboring a self-transmissible plasmid. Not surprisingly, group I Col factors are transferred most efficiently by the type of sex factor, either I-like or F-like, present in the strain in which they were originally found. Table 3 shows the frequencies of transfer of group I Col factors when transfer is promoted by various F-like and I-like plasmids (228). The transfer of ColE and ColK factors is promoted much more efficiently by I-like plasmids than by F-like plasmids. The exceptions are ColE1-16 and ColE1-K30 which are efficiently transferred also by F-like plasmids; ColE1-K30 is the only group I Col factor among those tested which was originally found in a cell harboring an F-like plasmid (86, 166, 167).

The products determined by the self-transmissible plasmids which enable them to promote the transfer of group I Col factors are unknown, but neither *recA*$^+$-dependent recombination between the two plasmids nor cotransfer of the self-transmissible plasmid is necessary. Transfer is unaffected by the host *recA* mutation and can also be promoted by a *traI* mutant of F which cannot transfer F DNA although the cells harboring the plasmid form sex pili (4, 478). The transfer of many small R factors is similarly promoted very efficiently by self-transmissible plasmids including group II Col factors (11, 174, 227, 358, 407).

TABLE 3. *Cotransfer frequencies of group I Col factors*[a]

Transfer promoted by F-like plasmids

Sex factor	Recipients receiving sex factor which have also received group I Col factor (%)		
	ColE1-16	ColE2-P9	ColK-235
F′*lac*	44	<0.01	<0.01
R1*drd*19	24	<0.01	<0.01
ColV-K94	60	<0.01	<0.01

Transfer promoted by I-like plasmids

Sex factor	R$^+$ recipients which are Col$^+$ (%)		
	ColE1-16	ColE2-P9	ColK-235
R144*drd*3	92	80	90
R538I*drd*1	90	93	92

[a] From Harden (228) and personal communication. *E. coli* K-12 donors harboring both a sex factor and a group I Col factor were mated for 20 min with a recipient to determine the frequencies of cotransfer of the group I Col factor.

COLICINS

Relationships Between Colicins

Colicins are usually purified from cultures which have been induced with mitomycin C to increase the colicin titer. Colicins purified from induced cultures are found to be proteins. But in earlier studies, colicins A-CA31, K-235, and V-357 were purified from uninduced cultures and were found to be proteins associated with lipopolysaccharide components of the cell envelope (17, 201, 280). Colicin K-235 was clearly shown to be associated with the specific O-antigen of the producing strain (201).

In fact, uninduced cultures of ColK$^+$ bacteria, and probably of other Col$^+$ bacteria, contain both forms of colicin (200, 309, 568). It is not clear whether the association with lipopolysaccharide is simply fortuitous or intrinsic to the mechanism of colicin release. But whatever the explanation, its significance from an ecological point of view is that it may increase the stability of colicin without inactivating it.

Colicins D-CA23, E1-K30, E2-P9, E3-CA38, Ia-CA53, Ib-P9, and K-235 have been isolated as simple proteins with molecular weights of between 45,000 and 90,000 (Table 4). Colicin M-K260, which was isolated from uninduced cultures, is exceptional; it consists of a polypeptide with a molecular weight of 18,000 which retains full biological activity only when associated with phosphatidylethanolamine to form a complex with a molecular weight of 27,000 (54).

Evolution of colicins E2-P9 and E3-CA38 from a common ancestor is indicated by their immunological cross-reactions and regions of similar amino acid sequences (252). Similarly, colicins Ia-CA53 and Ib-P9 show immunological cross-reactions, and the mapping of peptides derived from tryptic digests demonstrates that much of their amino acid sequence is similar (295, 346).

There are no immunological cross-reactions between colicin E1-K30 and colicins E2-P9 or E3-CA38, or between the E colicins and colicin K-235 (344, 505). There are no further indications of primary sequence relationships between colicins, but several colicins have similar physical properties. Calculations of frictional coefficients indicate that colicins are asymmetric molecules, with colicins E1-K30, K-235, Ia-CA53, and Ib-P9 having higher f/f_0 values than colicins E2-P9, E3-CA38, and D-CA23 (see 344). The colicins with higher axial ratios all impair the energized state of the cytoplasmic membrane, whereas colicins E2, E3, and D affect either DNA or protein synthesis. Other common properties of colicins include their hydrophilic nature, the absence of cysteine residues in colicins K-235, Ia-CA53, and Ib-P9 (390, 345), and the single cysteine residue per molecule of colicins E2-P9, E3-CA38, and E1-K30 (252, 505). Colicin D-CA23 is therefore unusual in having six cysteine residues per molecule (558). Colicins E2-P9, E3-CA38, Ia-CA53, Ib-P9, and K-235 have each been isolated in two or more forms which differ in electrical charge (185, 200, 252, 309, 344, 348).

Colicin Synthesis in Relation to Lysogeny

The synthesis of most of the proteins coded by temperate phage genomes is repressed

TABLE 4. *Physicochemical characteristics of colicins*

Colicin	Mol wt	Frictional coefficient (f/f_0)[a]	Isoelectric point (pI)	Cysteine residues	Reference
D-CA23	90,000	1.58	4.70	6	558
E1-K30	56,000	2.02	9.05	1	505
E2-P9	60,000	1.45	7.63[b]	1	252
			7.41		
E3-CA38	60,000	1.40	6.64[c]	1	252
Ia-CA53	80,000	1.82	10.50	0	346
Ib-P9	80,000	1.76	9.53	0	346
K-235	45,000	1.70	5.14	0	309
			5.21		
M-K260	27,000[d]				54
	18,000[e]				

[a] See Konisky (344).

[b] Colicin E2-P9 can be isolated in two interconvertible forms which have different isoelectric points.

[c] Multiple forms of colicin E3-CA38 with different isoelectric points have been described by Glick et al. (185).

[d] Biologically active form (includes phosphatidyl ethanolamine).

[e] Protein component of colicin M. Molecular weights of both forms were determined by polyacrylamide gel electrophoresis.

under many growth conditions. Lysogeny appears to have evolved in populations of temperate bacteriophages to maximize their reproduction since a "choice" may be made between two developmental pathways depending on the growth conditions of the host (139). Similar considerations may account for the repression of functions associated with the transfer of Col factors and other plasmids. Derepressed mutants of ColIb-P9, for example, may be at a selective disadvantage under many growth conditions because they decrease the growth rate of their host and bring about deleterious changes in its cell envelope (129).

The synthesis of colicins also appears to be repressed in most cells harboring Col factors. If colicin synthesis is a lethal event, derepression of colicin synthesis in a proportion of a clone of Col^+ cells may increase the chances of survival of the remaining members of the clone by inhibiting the growth of competing bacteria. At the moment, however, there is little ecological evidence upon which to base such a claim.

The formation of lacunae by a small proportion of cells harboring ColE or ColK factors, and micromanipulation experiments, which show that only a small proportion of pairs of Col^+ and sensitive Col^- cells are unable to coexist (456), both indicate that colicin synthesis is repressed in many Col^+ cells. Furthermore, the number of lacunae and the colicin titer both increase after UV irradiation. The possibility that all Col^+ cells synthesize small amounts of colicin all the time is, of course, difficult to exclude.

Cultures of bacteria harboring group II Col factors do not form such clear lacunae, but this is probably due to the increased proportion of cell-bound colicin and to the lower titers of colicin produced rather than to a fundamental difference in the mechanism of colicin synthesis (234). The synthesis of colicins determined by both group I and group II Col factors can be induced by mitomycin C (231).

The percentage of lacuna-forming cells (%LFC) in ColE2-$P9^+$ broth cultures varies considerably depending on the growth phase (230, 232). The %LFC remains at a constant minimum value of about 0.01% in exponentially growing broth cultures which have a colony count of less than 5×10^7/ml. When the colony count exceeds 5×10^7/ml, the %LFC increases rapidly more than 100-fold (Fig. 3). When a culture with a high %LFC is diluted into fresh medium and maintained at a density of less than 5×10^7/ml, the number of LFC per ml remains constant for several generations until the %LFC again reaches a minimum of about

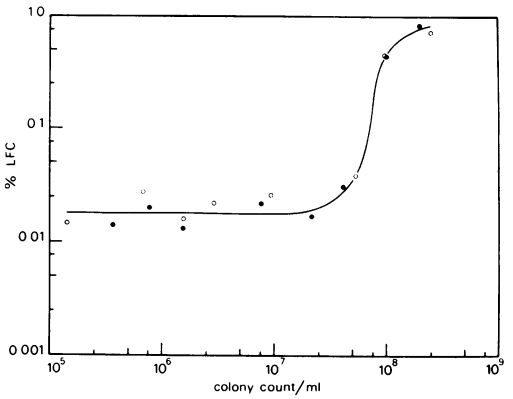

FIG. 3. *Increase in percentage of lacuna-forming cells (ordinate) with increase in colony count (abscissa). From Hardy and Meynell (232). An 18-h unshaken culture of E. coli K-12 strain AB1157 ColE2$^+$ was diluted 10^{-6} in broth. After 4.5 h of incubation, samples were removed at 30-min intervals for lacuna and colony counts. The results of two independent experiments are shown.*

0.01%. Cells releasing colicin exist for several hours but they do not divide to produce lacuna-forming daughter cells (230). Spontaneous induction of prophages varies similarly in batch cultures (381).

Ozeki et al. (456) suggested that cells which spontaneously produce colicin E2-P9 are not able to form colonies. A Col^+ culture was plated with indicator bacteria, and growth of the Col^+ bacteria was inhibited by chloramphenicol until lacunae were visible. The chloramphenicol was then removed to allow the Col^+ bacteria to grow, but no colonies formed in the centers of lacunae. However, the experiment is open to the criticism that chloramphenicol induces the synthesis of colicin E2 (28, 326).

The striking parallels between prophage induction and the induction of colicin synthesis suggests that a similar mechanism is responsible for both events. Many treatments which inhibit DNA synthesis bring about prophage induction and also induce the synthesis of colicins. (Synthesis of sex pili is not derepressed by these treatments [584]). Treatments which induce colicin synthesis include UV, X-irradiation, mitomycin C, thymine starvation, and heat treatment of *ts* mutants which rapidly stop DNA synthesis at 42 C (233, 236, 282, 297, 303, 343, 380, 382, 520).

However, unlike prophage induction, induction of colicin synthesis is not necessarily accompanied by enhanced replication of the Col factor. In *Proteus mirabilis*, the increase in ColE1-K30 DNA after mitomycin C treatment

parallels the increase in colicin titer (125). However, in *E. coli*, extensive replication is not a necessary condition for increased titers of colicins E1-K30, E2-P9, or Ib-P9 after induction, and induction is not accompanied by enhanced replication of the plasmid (137, 231, 297, 500).

The introduction of irradiated DNA into a colicinogenic cell or into a lambda lysogen induces colicin synthesis or the prophage (41, 42, 124, 251). DNA which can exist as an independent replicon produces much more efficient indirect induction of prophage lambda. For example, irradiated DNA of phage P1, F, ColIb-P9, and other plasmids are all effective (184, 414, 485), but irradiated DNA from phages T6 or ϕ80 does not indirectly induce lambda, and the DNA from an Hfr is much less effective (42). Colicin E2 synthesis is induced in *ts tif-1* mutants at 42 C (R. C. Oliver and K. G. Hardy, unpublished data).

The spontaneous and induced synthesis of colicins and lambda is inhibited by most *recA* mutations (27, 60, 76, 184, 233, 246, 254). The effects of the *lexA* (or *exrA*), *lexB*, *zab*, *uvrF*, *recF*, and *infA* mutations, all of which inhibit prophage induction (16, 69, 127), on colicin synthesis may provide further tests of the similarity of the control mechanisms operating in lysogenic and colicinogenic bacteria.

In view of the similarities between lambda prophage induction and colicin induction, the mechanism of prophage induction is of particular interest. It is known that the λ cI repressor is cleaved after induction by mitomycin C; a fragment with a molecular weight of 14,000 (about half the size of the repressor) can be detected (481). However, the sequence of events leading to the cleavage of the repressor is unknown.

Goldthwait and Jacob (203) proposed that an inducer, possibly a nucleotide derivative, accumulated in cells and inactivated the cI repressor. The same inducer was believed to accumulate at high temperature in the *tif-1* mutant, but no alterations of DNA synthesis or in nucleotide pools have been detected in this strain (336, 488), although the addition of various nucleotides does affect the level of spontaneous induction (337, 430).

Witkin (599) has suggested that the inhibition of DNA synthesis in strains which are *recA*$^+$ and *lex*$^+$, and in which there is no inhibition of protein synthesis, leads to the induction of several closely coordinated events such as prophage induction and filamentous growth. The induction of colicin synthesis can probably be added to this list of inducible functions. UV may have a similar effect on these processes because they may all involve repressors susceptible to a protease induced after the inhibition of DNA synthesis (481). An alternative view is that the repressors of λ and possibly of other inducible prophages and the postulated repressors of *col* genes on Col factors bind to nicked DNA; after induction by agents which damage DNA, less repressor may be available to act on specific operators (544).

Colicinogenic cells resemble some lysogenic cells in being more sensitive to inducing agents (251), but the reason for cell death after colicin induction is unknown. Inselburg (288) found that a ColE1-K30 plasmid with an insertion of about 600 nucleotides (of non-ColE1 DNA), which did not determine an active colicin protein, did not increase the sensitivity of cells to inducing agents. However, cell death does not appear to result from an overwhelming of the cell's immunity to colicin, since the death of ColE2$^+$ cells after induction is not due to lesions characteristically produced by that colicin, namely, the degradation of DNA (390).

Differences between λ prophage induction and the induction of colicin synthesis include the differing effects of inhibiting protein synthesis and the absence of zygotic induction when Col factors are transferred to Col$^-$ bacteria. The proportion of colicin-producing cells, as estimated by the lacuna technique, is markedly increased when protein synthesis is allowed to resume after a period of inhibition (28, 326). There is no zygotic induction when Col factors are introduced into Col$^-$ cells (421). It would be surprising if zygotic induction were to occur; there would seem to be a far greater selective advantage to Col factors which were immediately able to form a stable association with new hosts. Otherwise, the whole process of conjugation would simply be another way of killing cells which could be achieved more readily by the action of colicins.

As the selective advantage of colicinogeny is often assumed to be the release of colicin which then kills closely related bacteria (as well as the producing cell) to provide a selective advantage for other members of the Col$^+$ clone, it is interesting that much of the colicin produced is not released into the growth medium but remains cell bound (234). The most extreme example appears to be colicin M-K260 (54), where the very low titer of colicin produced is almost entirely cell bound. The retention of colicin by the producing cells is more marked with group II Col factors, at least when they are in *E. coli* K-12 strains which are colicin resistant (234). More than 80% of the colicin

produced by late-logarithmic-phase cultures of bacteria harboring ColB, ColI, or ColV factors was cell bound, whereas more than 50% of the colicin produced by ColE$^+$ and ColK$^+$ cultures was released into the growth medium. The effect of the *recA* allele on colicin synthesis is also correlated to some extent with the two groups of Col factors. The effect of this mutation is far greater on the synthesis of colicins determined by group I Col factors (234, 246). The titer of colicin E2-P9 is about 100 times lower in cultures of a *recA*$^-$ host. The titers of colicins V-K94 and V-K30 are the same in *recA*$^+$ and *recA*$^-$ hosts, but not all group II Col factors remain totally unaffected by the *recA* allele (234).

Synthesis of the immunity protein determined by ColE3-CA38 may also be induced by mitomycin C (304, 523). Jakes et al. (304) found that 50 to 100 times more E3 immunity protein could be isolated from cells treated with mitomycin C.

Little is known about the other products determined by group I Col factors, although several species of mRNA can be detected in minicells harboring the CloDF13 plasmid, which determines a bacteriocin and an immunity protein very similar to those determined by ColE3-CA38 (120, 121). In uninduced minicells, one species of RNA (about 21*S*) could be detected, together with proteins with molecular weights of about 70,000, 20,000, and 11,000 (351). Mitomycin C did not induce cloacin synthesis in minicells; however, in minicells harboring a mutant Clo plasmid which determined increased levels of colicin, three additional classes of mRNA (19.5*S*, 14*S*, 12*S*) and five additional proteins with molecular weights of 58,000, 44,000, 28,000, 16,000, and 14,000 could be detected (351).

EFFECTS OF COLICINS ON SENSITIVE BACTERIA

Analogous Systems

To kill sensitive cells of *E. coli* K-12, colicins must first bind to receptors in the outer membrane of the bacteria (stage I). Subsequent events (stage II) are apparently energy dependent; cells treated with uncoupling agents remain susceptible to rescue by trypsin which presumably destroys the colicin adsorbed to surface receptors. What happens to colicins after they have adsorbed to receptors is unknown. It seems likely, however, that they are either transported or rearranged on the surface so that they come into contact with the cytoplasmic membrane; several colicins disrupt the "energized state" of the membrane. Colicin E3-CA38 attacks ribosomes both in vivo and in vitro, so this colicin (or perhaps a part of it) probably penetrates the cytoplasmic membrane.

The effects of colicins may be compared with the actions of other proteins such as bacteriophages and their ghosts, which interact with both the cell wall and cytoplasmic membrane, sex pili, and competence factors.

Initial Contact with the Cell: Receptors

Analogies with bacteriophages originally led to the proposal that colicins adsorbed to receptors on the bacterial surface and that colicin resistance may result from the absence of functional receptors (155). The loss of these postulated receptors, which were believed to be reasonably specific for particular colicins, forms the basis of Fredericq's classification (156).

The relationship between the binding of colicins and bacteriophages to receptors was strengthened by the finding that resistant mutants were often insensitive to both a colicin and a bacteriophage, suggesting that they adsorbed to the same receptor. Cross-resistance studies suggested that common receptors existed for bacteriophage BF23 and the E colicins (157), bacteriophage T6 and colicin K (158), and bacteriophages T1 and T5 and colicin M (175).

Biochemical and genetic analyses have, in general, confirmed these assumptions about colicin receptors. The receptor for colicin E3-CA38 has been isolated by Sabet and Schnaitman (490). It is a glycoprotein with a molecular weight of 60,000 and, judging from its being released by Triton X-100 in the presence of ethylenediaminetetraacetic acid (EDTA), it is probably situated in the outer membrane. The purified receptor inactivated colicins E2-P9 and E3-CA38, but the equivalent glycoprotein isolated from a colicin-resistant mutant did not. The receptor did not inactivate colicin E1-K30; the authors suggested that other components of the cell envelope, in addition to the glycoprotein they isolated, may be necessary to form the receptor for colicin E1-K30. The carbohydrate bound to the protein appears to be necessary for colicin reception; periodate inactivated the molecule. The receptor for colicins E2-P9 and E3-CA38, specified by the *bfe* locus at 77.5 min (307) can also be distinguished genetically and physiologically from the receptor for colicin E1-K30. Hill and Holland (257) isolated mutants which could bind colicin E1-K30 but not colicins E2 or E3.

The receptor for colicins Ia-CA53 and Ib-P9, believed to be specified by the *cir* locus at about

41 min (67), could also be solubilized with Triton X-100 and EDTA (347). Similarly, the receptor for colicin K-235, specified by the *tsx* locus at 11 min, is trypsin sensitive and is probably situated in the outer membrane; it could not be detected in mutants resistant to colicin K (489, 587). Braun et al. (55) have isolated the receptor for colicin M-K260 and have shown that the binding of colicin M to the receptor prevents the binding of bacteriophage T5. Again, the receptor appears to be a protein (molecular weight about 85,000) in the outer membrane.

The binding of colicins and bacteriophages to common receptors raises the possibility of their common ancestry (160, 162), but no immunological cross-reactions can be detected between colicin K-235 and bacteriophage T6 (7), and Braun et al. (55) could not detect a protein in sodium dodecyl sulfate (SDS)-treated bacteriophage T5 which had a molecular weight similar to that of colicin M-K260.

Little is known about the binding of sex pili to recipients and there are no reports of colicin-resistant mutants being poor recipients when mated with either F-like or I-like donors. However, proteins in the outer membrane may also be important in the binding of sex pili; Skurray et al. (528) found that mutants which were unable to act as recipients for F^+ donors lacked an outer membrane protein.

Other reports also suggest that proteins in the outer membrane are crucial to the interaction of *E. coli* with its environment. The colicin E receptor is particularly interesting in this respect; it is also the receptor for vitamin B_{12}. The vitamin adsorbs to the receptor and is then transported into the cell in an energy-dependent reaction (126, 315). Cyanocobalamin prevents the formation of inhibition zones around colonies of $ColE2^+$ or $ColE3^+$ bacteria and colicin E1-K30 is a competitive inhibitor of vitamin B_{12} binding (126). The *btuB* locus, determining the vitamin B_{12} receptor, maps at the same position as the *bfe* locus which determines sensitivity to the E colicins (315). Taylor et al. (554) report a molecular weight of 22,000 for the B_{12} receptor of *E. coli* B, in contrast to the value of 60,000 found for the the colicin E receptor (490). (Vitamin B_{12} is not an essential requirement for *E. coli,* although there are at least two vitamin B_{12}-dependent pathways [73, 115]).

The $tonA^+$ and $tonB^+$ loci may also have been retained during the evolution of *E. coli,* despite the sensitivity they confer towards colicins and bacteriophages, because they also have a more beneficial role, the uptake of iron. A series of deletions may be isolated in the *tonB* region which are resistant to all or some of the following—bacteriophages T1 and $\phi 80$, and colicins B, I, M, and V; and *tonB* mutants are often trp^- (209, 210, 219). The $tonB^+$ gene product is also involved in the uptake of iron which is complexed with either citrate, enterochelin, or ferrichorme (226). The uptake of iron complexed with enterochelin occurs in several stages (581). Enterochelin (a trimer of 2,3-dihydroxybenzoylserine) is synthesized when the intracellular concentration of iron falls below about 2.2 mM (219). $TonB^+$ cells have a high affinity for complexed Fe^{3+} which is transported into the cell in an energy-dependent reaction. Mutants which are $tonB^-$ retain the active transport step, but have much less affinity for Fe^{3+}. Many $tonB^-$ mutants secrete excessive amounts of enterochelin which, incidentally, inactivates colicin B (219), presumably because they are unable to accumulate sufficient iron to repress its synthesis.

The proteins determined by the *tonA* and *tonB* loci often function coordinately (226). The $tonA^+$ gene product is the receptor for colicin M and bacteriophages T1, T5, and $\phi 80$, as well as being involved in the uptake of the ferrichrome-iron complex (226).

About 95% of the mutants of *E. coli* selected for resistance to colicin B-CA18 are deletions (9, 210, 299), whereas resistance to colicin E2-P9 can be brought about at high frequency by point mutations (57), suggesting that these colicins may interact with the surface in different ways.

The killing of cells by colicins follows single-hit kinetics (see Hedges [239] for a theoretical discussion). A lethal unit, the mean number of colicin molecules required to kill one cell, varies from 1 to several thousand (388, 413) in different experiments. The results are generally interpreted to demonstrate that one colicin molecule kills a sensitive cell with a certain probability, although it is worth remarking that single-hit kinetics in heterogeneous biological populations can result from very different forms of reaction (607).

The element of probability in the single-hit killing by colicins may result from a proportion of inactive colicin molecules or from a proportion of ineffective receptors. (The interpretation of survival curves may also be complicated by desorption of colicin from receptors which allows a molecule to attach to more than one cell during experiments designed to determine survival curves [511].) Variations in the proportion of "nonlethal" receptors may also account for biphasic survivor plots which are often found when sensitive bacteria are treated with colicins (71, 345, 388, 464, 510). For example, for 95% of cells killed by colicin Ia-CA53, a lethal

unit corresponds to 10 colicin molecules: for the remaining 3% of cells, about 200 colicin molecules constitute a lethal unit (345).

Little is known about the distribution of receptors on the cell surface, but several authors (see 489, 533) have remarked on the possibility that colicin receptors may be located at the junctions between the cell wall and the cytoplasmic membrane (20, 35). Bacteriophages T4 and ϕX174 are thought to attach to these sites and sex pili also seem to arise from them, so they might be general channels for the transport of large molecules through the cell envelope (20, 21, 22). There are about the same number of colicin E and vitamin B_{12} receptors on the cell surface (about 200 per cell in *E. coli* K-12 strain C600) as there are wall-membrane junctions (490, 589). There are more receptors, about 2,000 per cell, for colicins Ia-CA53 and Ib-P9 (345, 347), but some of these may be nonlethal receptors.

Effects of Colicins on the Cytoplasmic Membrane

What happens to colicins after they have adsorbed to receptors is largely unknown, but several studies suggest that they are rearranged or transported after adsorption in order to bring them into contact with the cytoplasmic membrane. Colicins E2-P9 and E3-CA38 are not transported by the vitamin B_{12} transport system, however (126). Regions of contact between the cytoplasmic membrane and outer membrane (504) may provide access to the cytoplasmic membrane for colicins, such as E1-K30 and K-235, which affect the energized state of the membrane.

Colicin receptors in the outer membrane are not essential for colicin action, provided that the colicins are able to penetrate the cell wall. This was first suggested by the results of Šmarda et al. (51, 532, 533) who found that stable L-forms of *Proteus mirabilis* and *E. coli* B were sensitive to colicins, despite their resistance to bacteriophages and the absence of detectable cell wall components. Bhattacharyya et al. (33) found that the accumulation of proline by membrane vesicles prepared from either colicin-resistant mutants or from colicin-sensitive bacteria were equally sensitive to inhibition by colicin E1-K30. However, a tolerant mutant (*tolC*), which retained the colicin E1 receptor although it was insensitive to the action of colicin E1, gave rise to vesicles which were colicin insensitive. Similarly, energy-dependent transport in spheroplast preparations derived from colicin K- or colicin A-resistant bacteria or from staphylococcin-resistant mutants of *Staphylococcus aureus* retained sensitivity towards the respective bacteriocins (313, 551). There is an analogous interaction of modified bacteriophages with spheroplasts derived from bacteriophage-resistant bacteria. Benz and Goldberg (30) prepared contracted bacteriophage T4 particles by treating them with urea or by temporarily adsorbing T4 bacteriophages, which were mutant in gene 12 (coding for short tail fibers [324]), to cells. T4 particles treated in this way could infect T4-resistant bacteria which had been treated with penicillin or with lysozyme and EDTA to disrupt the wall. The interaction with the cell wall receptor leads to the contraction to a form which can interact with the cytoplasmic membrane.

The stage which brings colicins into effective contact with the cytoplasmic membrane may be the energy-dependent step which is inhibited by uncouplers. Two stages can be distinguished in the interaction of colicins E1, E2, E3, and K with sensitive cells (109, 260, 391, 464, 465). At stage I, colicin has adsorbed to surface receptors but has not yet initiated a chain of events leading irreversibly to cell death. At this stage, most of the cells can be saved by adding trypsin which presumably digests the colicin which is adsorbed to receptors. Some of the colicin molecules then enter stage II, possibly a colicin-cytoplasmic membrane interaction, which leads to cell death and cannot be reversed by trypsin. Uncouplers of oxidative phosphorylation, 2,4-dinitrophenol or *p*-trifluoro-methoxy-carbonyl cyanide phenylhydrazone, inhibit the transition to stage II (465). The energized state of the cytoplasmic membrane is necessary for the transition from stage I to stage II (310).

The properties of certain colicin-tolerant mutants originally suggested that colicins interact with the cytoplasmic membrane. Tolerant mutants are defined as those colicin-insensitive mutants which adsorb colicin but which are not killed as a result. Many of them have properties in addition to colicin tolerance, such as sensitivity to dyes and detergents, which suggested that they might have altered cytoplasmic membranes (see 477).

There is no direct evidence for alterations of cytoplasmic membranes, however, and not all instances of tolerance may result solely from changes in cytoplasmic membrane proteins. Deletions which include the *tolC* or *tolAB* loci lack several envelope proteins (451, 484). CetB mutants, which are tolerant to colicin E2, also have an altered cell envelope composition (263). Chai and Foulds (72) found that a protein was absent from the outer membrane of *tolG* mu-

tants. DeGraaf et al. (122) found that both tolerant and resistant (receptorless) mutants had alterations in the proportions of the same envelope proteins.

Tolerant mutants provide an approach to exploring the possibility that special areas (or functions) of the cell envelope participate in the transfer of several different classes of macromolecules, including colicins. The two most promising mutations in this respect are the closely linked *tolA* and *tolB* loci and the *tonB* mutation, although it is not known whether the latter is a mutation to tolerance or resistance.

The *tolA* locus maps near *gal* at 17 min and brings about tolerance to colicins A, E1, E2, E3, and K (32, 256, 422, 438). There is also an interesting relationship between tolerance to these colicins and the mechanisms of infection of bacteriophages f1 and λ. A *tol*II mutation (423), which is equivalent to a *tolA* mutation (32), was found to inhibit the penetration of phage f1 DNA (534). Cells infected with bacteriophage f1 are also insensitive to colicins E1, E2, E3, and K (534, 608); the protein responsible seems to be that determined by the f1 gene III, the minor coat protein which attaches to the cytoplasmic membrane (608).

The *tolA* mutation also inhibits the action of the λ *S* gene product, which is required for the transport of the λ-coded endolysin (483). The latent period after temperature induction of λcI_{857} is increased in *tolB* mutants (483). However, λS^- mutants are able to form plaques on *tolA* or *tolB* mutants (32, 483).

A similar pleiotropy is shown by TonB strains, many of which are insensitive to colicins B, I, M, and V and resistant to bacteriophages T1 and φ80 (210, 458), in addition to being defective in the accumulation of Fe^{3+} (226). At the moment, therefore, colicins determined by the larger, self-transmissible Col factors appear to be related through their ineffectiveness against *tonB* mutants, whereas group I Col factors determine colicins which are inactive on *tolA* mutants.

The effects of colicins A, B, E1, K, Ia, Ib, and S.8 on sensitive cells are broadly similar in that many energy-dependent syntheses and transport processes are inhibited (14, 148, 149, 218, 304, 372, 377, 420). Although the effects of these various colicins differ in detail, they are consistent with a primary action on the cytoplasmic membrane. The mechanism of action of these colicins has been discussed recently by Luria (378).

Colicins E1-K30 and K-235 uncouple several energy-dependent transport processes, such as the transport of amino acids (148). Luria (378) has suggested that the loss of amino acids may be responsible for the inhibition of protein synthesis in colicin E1-treated cells. The membrane is not completely disrupted; β-galactosidase is not released and the cells may also continue to accumulate α-methylglucoside through the phosphoenolpyruvate-phosphotransferase system (148). Unlike cells treated with classical uncouplers of oxidative phosphorylation, colicin E1-treated cells do not become more permeable to H^+ (148). However, K^+ is released and the cells also become more permeable to Mg^{2+} and Co^{2+} (109, 146, 258, 379, 588). The adenosine 5'-triphosphate (ATP) level also falls in colicin K-treated cells, but studies using *uncA* mutants suggest that, in cells which have a functional membrane-bound adenosine 5'-triphosphatase (ATPase), this is because the cells seek to maintain the concentration gradients of K^+ (and possibly of other cations) through the expenditure of ATP (466). The fall in ATP level per se is not responsible for the action of colicin E1-K30. Thus, when the decrease in ATP concentrations which normally accompanies the action of colicin E1 is inhibited by dicyclohexylcarbodiimide, an inhibitor of membrane-bound ATPase, the colicin still inhibits protein synthesis (146).

If the primary action of colicins E1 and K is to act as K^+ ionophores similar to valinomycin, our understanding of the other effects of these colicins may depend critically on the importance of K^+ and other cation gradients in maintaining the energized state of the membrane. An interpretation of colicin action within the framework of the chemiosmotic hypothesis of Mitchell may provide the most satisfactory explanation, since this model links the transport of substances across the membrane most directly with ionic gradients (412). However, little is known about how the transport of various metabolites is linked to K^+ gradients in *E. coli* (235).

Comparable Effects of Other Proteins

Colicins are not the only proteins capable of killing *E. coli*. Of the other proteins with this capacity, bacteriophage tails and sex pili are particularly interesting in relation to colicins because there is also a specific immunity to the effects of these proteins.

Ghosts of the T-even bacteriophages, which lack DNA, can be produced from bacteriophage particles by osmotic shock (9, 249). They resemble colicins such as E1-K30 in inhibiting many energy-dependent reactions in bacteria and, moreover, the killing follows single-hit

kinetics (132). The synthesis of protein, RNA, and DNA is inhibited in ghost-treated cells (38, 132, 133, 143, 176, 180, 250). The accumulation of several carbohydrates, including thiomethyl-β-galactoside, α-methylglucoside and glucose-6-phosphate is also inhibited, and potassium is released from cells treated with either UV-inactivated bacteriophage or bacteriophage ghosts (512, 525, 598). The interactions between bacteriophages or colicin E1 and the cell surface can be detected by an increase in the fluorescence of fluorescent probes (8-anilino-1-naphthalene-sulphonate or *N*-phenyl-1-naphthylamine) bound to the cell surface (56, 97, 98, 226).

Some of these effects of bacteriophage ghosts may be similar to those produced by intact bacteriophage particles on sensitive cells; RNA and DNA synthesis is inhibited even when bacteriophage-directed protein synthesis is inhibited. There is also a short-lived efflux of K^+ after adsorption of intact bacteriophage particles to cells capable of protein synthesis (512, 525). Bacteriophage-coded proteins presumably reverse these inhibitory effects after the phage DNA has entered the cells.

However, there are indications of qualitative differences between the actions of the tail proteins of intact bacteriophage particles and those of bacteriophage ghosts. Bacteriophage ghosts produce a far greater inhibition of macromolecular syntheses and transport processes (132, 133, 598). The cells lose ATP, suggesting nonspecific damage to the cytoplasmic membrane, which may be responsible for the inhibition of many energy-dependent processes (598).

Thus the effects of bacteriophage ghosts also appear to be more severe than those of colicins E1 or K, for example, which do not inhibit the transport of α-methylglucoside or cause the release of ATP. But at high concentrations, several colicins may lyse cells (70), and colicin M-K260 produces a more general increase in cell permeability than colicin E1 (54).

Although the inhibition of energy-dependent reactions in ghost-treated cells may be largely due to the loss of ATP, a further mechanism is suggested by studies on the ribosomes of ghost-treated cells (143, 180, 527). The 30S ribosomal subunits are inactivated through the degradation of 16S ribosomal RNA (rRNA). Pyocin R1, which resembles a contractile bacteriophage tail, also inhibits macromolecular synthesis by inactivating bacterial ribosomes (322, 323).

The effects of sex pili (strictly speaking, only an effect of donor bacteria has been demonstrated) on bacteria are strongly dependent on multiplicity. Recipients are killed only when mated with large numbers of donors (with a donor-to-recipient ratio of about 20:1), although there are bacteriostatic effects at lower multiplicities. The lethal effects of a high multiplicity of donors have been reported several times (5, 85, 213, 421), and the mechanism has recently been investigated in detail by Skurray and Reeves (529, 530, 531).

Lethal zygosis (5) can be demonstrated on agar plates and in aerated liquid cultures (530). "Stationary-phase" recipients are more sensitive than recipients in other growth phases. Hfr strains are much more effective than F^+ bacteria in producing lethal zygosis; the explanation may be similar to that suggested for the more severe effects of bacteriophage ghosts, namely a reversal of the inhibitory action once the complete F plasmid has entered the cells.

Again the effects of a high multiplicity of donors are more severe than those of most colicins. There is a less specific increase in cell permeability. The transport of galactosides is inhibited and β-galactosidase is released into the medium (530).

The initial stages of the interaction of competence factors with cells has certain parallels with the action of colicins. The pneumococcus activator has a molecular weight of 10,000 (563) and that produced by *Streptococcus* group H has a molecular weight of 5,000 (369, 453, 462). Macromolecular synthesis is inhibited during the development of competence in *Bacillus subtilis* (128) and in a group H *Streptococcus* (269, 270), although less inhibition was found in streptococci after the bacteriocin was removed from preparations of the competence factor.

The two stages which can be distinguished in the binding of streptococcal or pneumococcal competence factors resemble those which have been described for colicins. Adsorption of streptococcal competence protein is initially trypsin sensitive and is followed by a transition to trypsin insensitivity which leads to competence (369). The transition is believed to result from the passage of the competence factor through the cell wall to form a complex with the cytoplasmic membrane (369, 463).

Similar stages can be distinguished for the pneumococcal competence protein. Initial adsorption to the cell envelope is not dependent on metabolic activity, in contrast to the energy-dependent penetration of the competence factor which then binds to receptors on the cytoplasmic membrane (561, 606).

An energy-dependent stage can also be distinguished in the killing of *Pasteurella septica*

by antibody and complement; Griffiths (211) has pointed out the similarities to the protection afforded by uncouplers against the action of colicin K, for example (465).

Action of Colicins E3-CA38 and E2-P9

Colicins E2-P9 and E3-CA38 have clearly evolved from a common ancestor so it is all the more remarkable that they have such strikingly different effects on sensitive cells.

Colicin E3-CA38 affects rRNA and hence protein synthesis, both in vivo and in vitro. Its action on ribosomes in vitro suggests that colicin E3, or possibly some part of it, enters cells. The mechanism of action of this colicin has been recently reviewed by Bowman et al. (48) and Nomura (434).

Colicin E3-CA38 brings about the cleavage of a fragment about 50 nucleotides long from the 3′ end of 16S rRNA (46, 507). Purified colicin E3 must be used to demonstrate the in vitro action since crude preparations contain sufficient immunity protein to inhibit colicin action (39, 47). Even highly purified colicin E3 was found by Jakes and Zinder (306) to contain immunity protein tightly bound to the colicin in equimolar amounts. Colicin E3 which is completely free from immunity protein has a greater in vitro activity (306).

The importance of colicin receptors in determining sensitivity to colicin E3 is illustrated by the finding that it also cleaves a fragment from the 16S rRNA of ribosomes from *Bacillus stearothermophilus* or *Azotobacter vinelandii* (522), although the bacteria themselves are colicin resistant (3′ terminal sequences of 16S rRNA are known to be similar in many prokaryotes [600]). Turnowsky et al. (569) have reported that colicin E3 also inactivates mouse ribosomes in vitro, but yeast ribosomes are unaffected (522).

Both 30S and 50S ribosomal subunits must be present for colicin E3 to have any effect on ribosomes in vitro, although only the 30S subunit is inactivated (40, 45). A requirement for a particular ribosomal conformation is also consistent with the inhibitory effects of streptomycin on the in vitro action of colicin E3 (104), although this might equally indicate that both antibiotics act on closely related sites on the ribosome (549). Colicin E3 does not appear to activate a ribonuclease attached to the ribosomes (400).

The action of cloacin DF13, determined by a plasmid found in *Enterobacter cloacae* DF13, is so similar to that of colicin E3-CA38 that it would be very surprising if they had not evolved from a common ancestor. Cloacin DF13 also cleaves a fragment about 50 nucleotides long from the 3′ end of 16S rRNA, both in vivo and in vitro (121). The amino acid compositions of cloacin DF13 and colicin E3 are very similar, although the two bacteriocins can be distinguished by their action on various indicator bacteria (119, 542). Cloacin DF13 is inactive against the ribosomes of *Bacillus licheniformis* or *Saccharomyces carlsbergensis* (121). Colicin D-CA23 also specifically inhibits protein synthesis, but the amino acid composition of this colicin is very different from that of colicin E3 and it is not known whether this colicin also affects rRNA (558, 560).

In marked contrast to the effects of colicin E3, colicin E2-P9 and other E2 colicins (476) bring about the degradation of DNA and also inhibit cell division (31, 260, 431). The DNA appears to be degraded by the combined action of endonucleases, which give single- and then double-strand breaks, followed by an exonuclease attack (261, 441, 480). No known nucleases have been unequivocally implicated in the process (261, 441). Almendinger and Hager (6) reported that there was much less DNA degradation in mutants lacking endonuclease I which were treated with colicin E2-P9. But Obinata and Mizuno (441), Holland and Holland (261), and Buxton and Holland (65) found that these mutants were not less sensitive to colicin E2.

Purified colicin E2-P9, presumably lacking any protein equivalent to E3 immunity substance, has no nuclease activity in vitro, although the T_m is lowered (480). However, comparable experiments with colicin E3 suggest that the precise conformation of the DNA (or perhaps of RNA attached to the DNA), as well as complete removal of any immunity protein, may be critical to any demonstration of colicin E2 action in vitro. It is not known whether colicin E2 penetrates cells. It is less effective against λ-lysogens; the product of the *rex* gene, which is known to affect the membrane in lysogens infected with T4rII or T1, is responsible (498). Similarly, the protection conferred by φX174, even when inactivated by UV, may result from alterations of the envelope produced by the bacteriophage (508).

Immunity to the Effects of Colicins, Bacteriophages, Bacteriophage Ghosts, and Sex Pili

Bacteriophage genomes, transmissible plasmids, and Col factors code for a specific immunity to the effects of proteins which they determine. Little is known about the mechanisms of this protection except that which is

determined by ColE1-K30 and CloDF13. In each case, however, the immunity can be overcome by high concentrations of the agent in question.

The immunity conferred by Col$^+$ strains is highly specific; ColIb-P9 and ColIa-CA53, for example, determine colicins which are very similar (295, 346) but they confer immunity only to the colicin of the corresponding type, Ia or Ib (539). T-even bacteriophages are immune to superinfection by the same or closely related bacteriophages. This superinfection exclusion prevents entry of the bacteriophage DNA; the DNA is released after adsorption of the bacteriophage, but it is degraded outside the cytoplasmic membrane (except in mutants lacking endonuclease I) (10, 135, 574). Similarly, F$^+$ cells are immune to lethal zygosis (531).

The immunity determined by T-even bacteriophages is less specific than colicin immunity in the sense that bacteriophage T6, for example, determines immunity to the effects of T2, T4, and T6 ghosts, but not to the actions of bacteriophages T3 or T7 (447, 590). Bacteriophage T6 and colicin K adsorb to the same receptor (489, 587), but the similarity does not extend to their specific immunity reactions; infection with bacteriophage T6 does not protect the cells from the action of colicin K (572).

The specificity of the immunity determined by sex factors towards lethal zygosis is likely to be the same as the specificity of the surface exclusion they determine.

The proteins responsible for the immunity towards colicin E3-CA38 and cloacin DF13 have been purified (120, 304, 523, 524). They are both acidic proteins with molecular weights of about 10,000. Sidikaro and Nomura (524) found that the immunity protein was synthesized in vitro when ColE3-CA38 DNA was used as a template in a protein synthesizing system. There are indications that ColE2-P9 may code for a polypeptide analogous to the E3 immunity protein (304).

The mechanism of protection by the colicin E3 or cloacin DF13 immunity proteins is unknown, but in both cases 1 mol of immunity protein neutralizes 1 mol of bacteriocin (306) when the colicin is assayed by the in vitro inactivation of ribosomes. But sensitive cells treated with a mixture of immunity protein and colicin are killed even when a 300-fold excess of immunity protein is present in the medium, so the adsorption of colicin to receptors in the outer membrane is presumably unaffected by the immunity protein (120, 304, 523). Colicin-immune cells adsorb as much colicin as sensitive cells (345, 388). Although cells harboring ColE1-K30 adsorb colicin E1, there is no increase in the fluorescence of fluorescent probes, an effect normally seen after the addition of colicin E1 (97, 464).

Production of colicin immunity protein is presumably constitutive; all Col$^+$ cells are immune. But the synthesis of the immunity protein is also induced by mitomycin C (304, 523).

The proteins responsible for the immunity of bacteriophage-infected cells to bacteriophage ghosts have not been identified. Several new proteins were found in the cell envelope fraction after disc gel electrophoresis (152, 468). Two T4 bacteriophage genes, *imm* and *s*, are necessary for maximum immunity to superinfection or to the effects of bacteriophage ghosts (74, 94, 95, 448).

The determinant for immunity to lethal zygosis is probably close to the *tra* genes. If this were the case, it would be a distal marker so that recipients resulting from interrupted Hfr matings would lack the immunity determinant. Skurray and Reeves (529, 531) showed that Hfr strains were far more effective in producing lethal zygosis than F$^+$ strains. Immunity may be determined by the *traS* gene (surface exclusion) which maps between *traG* and *traD* (592).

Immunity directed against colicins or against bacteriophage ghosts appears to a "stoichiometric" reaction. Fredericq (161) found that immunity conferred by Col factors, and particularly by ColV plasmids, could be overcome by high concentrations of colicin. Levisohn et al. (372) found that 50 times more colicin Ib-P9 was required to kill a cell harboring ColIb-P9 than a Col$^-$ cell.

The immunity of cells infected with bacteriophage T4 against killing by bacteriophage ghosts is also believed to be a stoichiometric, rather than a catalytic, reaction (447, 573). Immunity to lethal zygosis is reduced in F$^-$ phenocopies (531), suggesting that surface exclusion and immunity are different aspects of the same mechanism. Bacteria harboring derepressed mutants of R factors may grow more slowly (129) because they produce so many sex pili that the surface exclusion mechanism is overwhelmed, and they consequently become susceptible to "lethal zygosis."

ECOLOGY OF COL FACTORS AND COLICINS

A great deal is known about the chemistry, synthesis, and mechanism of action of colicins.

But why do so many enterobacteria produce them? In other words, what is the selective advantage of colicin production conferred on either bacteria or plasmids which has led to the evolution and survival of Col factors?

The most plausible selection pressure is the advantage gained by enterobacteria which are able to kill other strains in an ecological niche where there is intense competition between closely related species. However, by no means have all investigations demonstrated that there are selective advantages conferred by colicinogeny on enterobacteria in their natural habitat. In examining the evolution and ecology of colicinogeny, it is important to consider what is being selected. Is it the *col* gene, the Col factor, a segment of the Col factor, or the colicinogenic clone of cells? Without further studies it is difficult to assess the relative importance of these "units of selection" (373).

In many ecological studies, there has been too much emphasis on the survival of the Col^+ clone and too little on the survival of the Col factor. For example, a Col plasmid will presumably increase its own chance of survival by enabling its host to kill equally or less competitive bacteria. In the presence of a more competitive host, possibly a strain with a higher growth rate in the prevailing conditions, the best strategy for a Col factor would be to transfer its allegiance to what is likely to become a dominant strain. The extent to which such strategies have evolved remains to be established.

Methods of Study

Three approaches to the problem of the ecological significance of colicinogeny can be distinguished. Competition experiments can be made between pairs of strains in germfree animals. Alternatively, investigations can be made of the characteristics of the different enterobacteria which succeed each other in colonizing the intestines of normal animals or man. Such analyses may indicate, for example, whether "transient" or "resident" bacteria are most likely to be colicinogenic or colicin resistant. Thirdly, the characteristics of pathogenic bacteria and the intestinal strains accompanying them can be analyzed with respect to colicinogeny.

Investigations Using Gnotobiotic Animals

Ikari et al. (283) fed germfree mice with a culture of paracolon strain CA62 producing colicins E1 and I, followed immediately by a culture of *E. coli* K-12 (Row) which is colicin sensitive. Both strains were found in the intestine for a week at the same concentrations found in mice which were inoculated with only one of the two strains. The results were not affected by the order in which the two strains were fed to the mice. Similar studies over 4 weeks showed that the strains still coexisted; the colicin-sensitive strain tended to predominate (Fig. 4). Furthermore, even when a large inoculum (2×10^7/ml) of the colicinogenic strain was allowed to become established in mice for 2 days, an inoculum of only about 10 bacteria of the colicin-sensitive strain was sufficient to allow this strain to become established in the intestine. About 1 to 10% of *E. coli* K-12 (Row) became colicinogenic. The proportion of bacteria which became colicin resistant throughout the 4-week period was not recorded.

Kelstrup and Gibbons (325) fed germfree mice with *E. coli* K-12 $ColE2^+$. (Presumably only the nonconjugative plasmid, ColE2-P9, was present in the strain so that there should be no transfer of Col factors during the experiment, although this was not explicitly stated by the authors.) After 1 week, the mice were caged with one of two other groups of germfree mice. One group had been infected per os a week before with *E. coli* K-12 strain W3110 (colicin

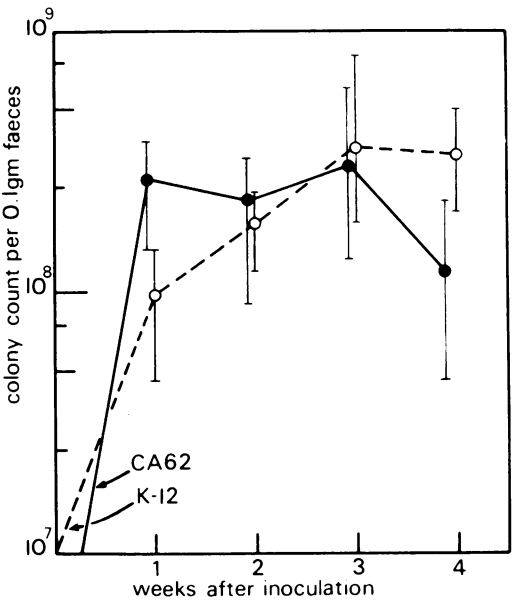

FIG. 4. *Coexistence of a colicinogenic strain, paracolon strain CA62 (ColE1, ColI), with a colicin-sensitive strain, E. coli K-12 (Row), in germfree mice (from Ikari et al. [283]). Thirty germfree mice were inoculated per os with 2×10^7 cells of E. coli K-12. Drinking water was inoculated with 10^8 cells of the colicinogenic strain per ml. The vertical bars indicate the range of bacterial counts at each sampling time.*

sensitive), and the second group had been infected with an E2-resistant mutant of W3110. Fecal samples demonstrated that the colicinogenic strain gradually became predominant in both groups of mice, comprising more than 90% of the bacteria after 35 to 44 days. The colicinogenic bacteria became predominant in the mice infected with the colicin-resistant strain, so any selective advantage due to the presence of the Col factor could not be ascribed to the killing of the coexistent bacteria. Similar results were obtained by Craven and Barnum (99) in gnotobiotic pigs. The competing bacteria in this case were a colicinogenic strain of *E. coli* isolated from a healthy pig and which was known to be a dominant strain in a group of pigs, and a second strain, a hemolytic *E. coli*, which was sensitive to the colicin produced by the first strain. A colicin-resistant mutant of the hemolytic Col^- strain was also used. The colicinogenic strain was found to predominate (comprising 80 to 90% of the total) in the pigs, irrespective of which strain was first added to the animal's food. Again, the Col^+ strain predominated in competition with the colicin-resistant mutant. The extent of Col factor transfer, if any, was not reported.

A possible explanation for the absence of any marked effect of colicin on colicin-sensitive strains in these experiments is that colicins are inactivated by proteases in the intestine. Kelstrup and Gibbons (325) reported that colicins A, B, D, E1, E2, I, K, and V were inactivated by mouse and human intestinal contents. The anaerobic conditions in the intestine may also decrease the effectiveness of colicins; anaerobically grown cells are insensitive to colicins E1 and K and to cloacin DF13 (56; Levisohn cited in 118, 377). Colicins are produced under anaerobic conditions (252, 394). However, nothing is known about colicin sensitivity or colicin synthesis at generation times of several hours which are characteristic of bacteria in the intestine.

Succession of Strains in the Intestine

Strains of *E. coli* in the intestine may be classified into resident types, which persist for several months, and transient types, which survive for much shorter periods (often only a few days) (506). Several investigations have been made to determine whether colicin synthesis is correlated with the ability of *E. coli* strains to persist in the intestines of man and animals for long periods.

Investigations on the succession of strains in the human intestine in relation to colicinogeny have been made by Branche et al. (50), Cooke et al. (91), Hettiaratchy (255), Emslie-Smith (141) and Sears et al. (506). The results are not in complete agreement.

Sears et al. (506) found that the inhibitory activity on agar plates of *E. coli* strains against other *E. coli* strains was not correlated with their length of survival in the intestines of four persons. More detailed investigations were made by Branche et al. (50) and Emslie-Smith (141), who examined individuals for longer periods and found a significant correlation between period of residence and colicinogenicity of strains. Both found that resident serotypes were more likely to be colicinogenic than were transient types and Emslie-Smith found also a significant correlation between colicin resistance and residence time. However, the findings of Branche et al. (50) that the presence of many serotypes was correlated with a low incidence of colicinogeny was not confirmed by Hettiaratchy (255).

In examining the fate of ingested broth cultures of *E. coli* in healthy persons, Cooke et al. (91) reported eight instances in which colicinogeny was apparently responsible for either the establishment of a strain or the displacement of a strain. In the same series of experiments, two instances were recorded where an ingested colicinogenic strain failed to displace a colicin-sensitive resident serotype, although on one of these occasions the resident serotype was found to have become colicin resistant. On two occasions, a colicin-sensitive strain became established despite the presence of a colicinogenic strain, although in both instances inhibitory activity could be demonstrated only after 48 h of incubation of colonies on agar plates. On two further occasions, a colicin-sensitive *E. coli* became established despite the presence, albeit transient, of colicinogenic bacteria. In general, these results suggest that colicinogeny is a significant factor in the succession of strains in the intestine, but that neither strains which produce colicin of low activity nor colicinogenic transient strains influence this succession.

Similar investigations made on the succession of strains in mice (176) and pigs (99, 545) are also not in full agreement but indicate that colicinogenic strains tend to predominate. Investigations by Willinger and Trčka (597) indicate that the selective advantage of colicinogeny may vary with the age of the animal. Three groups of healthy piglets were examined from birth for 9 weeks. After 9 weeks, 43% of the piglets had feces in which colicinogenic strains could not be detected compared with only 4% after 1 week.

Further data are needed for a more accurate

assessment of the importance of colicinogeny in the succession of enteric strains in animals and in man. A more promising approach to determining the significance of colicinogeny would be to follow up the monitoring of strain succession with further studies on those strains which become residents or transients apparently because of the presence or absence of a Col factor, or because of their colicin resistance or sensitivity. Colicin-sensitive transients could be made resistant to the colicins produced by resident strains and then reintroduced. Transient strains could also be made colicinogenic for one or more colicins. Conversely, Col^+ strains which tended to predominate could be cured of their Col factors and introduced into other animals. Germfree animals may be particularly useful in following up examples of succession if two strains showed the same relationship, with respect to residence, in these animals as in normal animals. Determination of the frequency of Col factor transfer should be an important aspect of all ecological investigations related to colicinogeny.

Colicins and Disease

The possibility that colicinogeny may be a factor in the establishment of bacteria in the intestine raises questions about whether pathogens are more likely to be colicinogenic than nonpathogens or whether a colicinogenic resident flora may prevent the establishment of a pathogen. An assessment of colicinogeny in this respect may be difficult because other factors which may contribute to pathogenicity, notably toxin production and drug resistance, may be determined by genes on a Col plasmid. This probably explains the association of bacteriocinogeny with pathogenic staphylococci of phage type 71 and the correlation of pesticinogeny with virulence in *Pasteurella pestis* (61, 107). Furthermore, in surveys designed to estimate the incidence of colicinogeny among commensals and pathogens, epidemiological relationships among strains, and still more among their plasmids, are often unknown.

In animals. The fact that Gratia's original strain, *E. coli* V which produced "principe V," was so designated because of its virulence for guinea pigs and rabbits, adds interest to the recent finding by Williams Smith (536) that increased invasiveness of *E. coli* strains in chickens is closely associated with the $ColV^+$ character. On finding that a plasmid which increased the invasiveness of *E. coli* strains determined colicin V synthesis, Williams Smith examined several other ColV factors isolated from cases of bacteremia in chickens and man. These Col factors were transferred to *E. coli* K-12 to determine whether they increased mortality when the bacteria were injected intravenously into chickens. All of the seven epidemiologically unrelated strains were found to harbor ColV factors which significantly increased the number of deaths produced by *E. coli* K-12 in chickens. The presence of ColE or ColI factors in *E. coli* K-12 did not increase the number of deaths (Table 5).

As Williams Smith points out, the role, if any, of colicin V in this remains to be elucidated. Purified preparations of colicin V (devoid of endotoxin) are nontoxic for mice (53, 238). In addition sonic extractions of the $ColV^+$ strains did not kill the chickens (536), although sonic extraction removes much of the cell-bound colicin V (234).

$ColV^+$ bacteria were shown to be less rapidly removed from the blood and peritoneal fluid than Col^- strains and, moreover, they survived longer in chicken serum (536). The mechanism of this enhanced survival is unknown, but it could be due to a change in the cell envelope determined by the Col factor, perhaps colicin immunity or sex pili.

Heller and Williams Smith (247) found that colicinogenic strains of *E. coli* were more common among pathogenic bacteria isolated from chickens than among commensal strains from the same source. They found that 152 out 173 (88%) *E. coli* strains were colicinogenic compared with 78 out of 168 (46%) strains isolated from healthy chickens.

Similar investigations on *E. coli* strains isolated from pigs do not indicate a correlation between colicinogeny and pathogencity in strains from these animals. Vasenius (576) found that 52% of 435 pathogenic strains were colicinogenic compared with 17% of 354 strains isolated from healthy pigs. However, Šarmanová and Salajka (495, 497) found that only 34% (243 out of 706) pathogenic (hemolytic) strains were colicinogenic compared with 132 out of 270 (48%) of nonpathogenic strains. Similarly, De-Alwis and Thomlinson (117) found a high proportion (44%) of colicinogenic strains among 231 commensal *E. coli* strains isolated from pigs.

In man. The incidence of colicinogeny in pathogenic strains isolated from man gives little ground for supposing that there is any overall relationship between colicinogeny and pathogenicity. The incidence of colicinogeny among pathogenic strains of *E. coli* isolated from children with gastroenteritis varies from 27 to 40% in different investigations, and the values among *E. coli* commensals vary from 5 to 100% (see 51). However, it is difficult to make compar-

TABLE 5. *Lethality for chickens of E. coli K-12 harboring ColV and other plasmids from different enterobacteria*[a]

Plasmids introduced into *E. coli* K-12	Donors used to introduce plasmid into *E. coli* K-12	Source of donors	No. of deaths after infection with the *E. coli* K-12 test strains[b]
ColV, ColE1, ColIa	*E. coli* H261	Human	27
ColV, ColIa	*E. coli* H247	Human	27
ColV, ColIa	*E. coli* F157	Chicken	26
ColV, ColIb	*E. coli* F107	Chicken	21
ColV	*E. coli* F120	Chicken	26
ColV	*E. coli* P72	Pig	27
ColV	*E. coli* F70	Chicken	25
ColE1, ColIa	Paracolon		5
ColIa	Paracolon		2
ColIb	*E. coli* F120	Chicken	2
ColI	*S. typhimurium*		4
ColE1	*E. coli* F105	Chicken	1
ColE1, F	*E. coli* B165 F^+	Cattle	4
ColE1	*E. coli* B165 F^+	Cattle	4
F	*E. coli* K-12 F^+		1
None			3

[a] From Williams Smith (536).

[b] Bacteria (3.0×10^9) from soft agar cultures were injected (intravenously) into 2-week-old chickens. Thirty chickens were injected with each of the Col^+ *E. coli* K-12 test strains.

isons between different investigations because different colicin indicators have been used and different precautions have been taken to exclude inhibition by agents such as bacteriophages and hydrogen peroxide.

In view of the recent findings by Williams Smith (536) on the increased invasiveness of $ColV^+$ strains, further investigations on the incidence of particular Col factors among human pathogenic strains would seem warranted. Fredericq and Joiris (159, 173) found that colicins B and V were more frequently produced by the dominant intestinal flora in cases of infection by *Salmonella paratyphi* B and *Salmonella typhi* than by the flora of healthy individuals.

An interesting relationship between the course of (bacterial) intestinal infections and changes in the colicinogeny of the intestinal flora has been noted several times (159, 177, 221, 553). In several cases it was found that the percentage of Col^+ strains among the commensal flora accompanying the pathogen increased during the course of dysentery infections. Whether the increase is due to alterations in the conditions in the intestine to favor existent Col^+ strains or, as seems more likely, to the transfer of Col factors from the pathogen to the accompanying enterobacteria could be established by investigating the colicin types of the pathogen and accompanying bacteria. Transfer from the pathogen is consistent with the observation made by Fredericq (159) that many of enterobacteria accompanying infections by *Salmonella paratyphi* B which determined colicin B synthesis also became colicinogenic for colicin B. The fact that the pathogen itself and several different strains of accompanying enterobacteria all produced the same colicin strongly suggests that transfer occurred at high frequency from the pathogen, particularly since $ColB^+$ strains could not be detected in stools recovered 25 days before the onset of disease. Tatarinova (553) found that colicinogenic enterobacteria accompanying *Shigella* infections were more common when the pathogen itself was Col^+. It was also found that, out of 193 cultures of *Shigella sonnei* examined, colicinogenic strains were more commonly associated with protracted and more severe cases of bacillary dysentery than Col^- strains.

The inactivation of colicins by proteases and their immunogenicity suggest that the prospects for the therapeutic use of colicins may be limited. However, it would be premature to conclude that none of them has potential therapeutic value, particularly in view of the increasing numbers of drug-resistant gram-negative bacteria which are responsible for infections. Attempts have been made to use colicins, or even colicinogenic bacteria, therapeutically. Studies by Nissle in 1916 (428) on the antagonism between strains of *E. coli* led to the introduction of a commercial preparation of *E. coli* Mutaflor (429), producing colicin X (459), which was used for many years. More recently, a similar approach has been described using *E. coli* strain M-17 (Colibacterin) for the treatment of

bacillary dysentery in the Soviet Union (for example, see 15). The bacterial preparation may be incorporated in gelatinous capsules for protection from the action of intestinal contents. Avanesova et al. (15) reported that the administration of Colibacterin for 3 to 4 weeks was a useful treatment for cases of dysentery due to *Shigella sonnei* and also reduced the length of the carrier state during convalescence.

Significance of Colicins Outside the Intestine

Inactivation of colicins may be less likely to occur outside the intestine, notably in the blood, urine, or peritoneal cavity. A strain of *Shigella* was rapidly eliminated by a colicinogenic strain of *E. coli* after the two strains had been injected into the peritoneal cavity of mice (177). The elimination of the *Shigella* strain coincided with an increase in the colicin titer in the peritoneal cavity. Colicin was also detected in the serum after intraperitoneal injection of Col^+ bacteria, even when there was no invasion of the bloodstream (222).

Colicin activity was detected in the serum of 30% of patients with urinary tract infections due to colicinogenic *E. coli* (202). In fact, serum appears to increase the bactericidal activity of colicins (7, 53, 575). Colicin activity can also be demonstrated in the blood of mice and rats after subcutaneous injection (53, 202).

Colicin V can also be detected in the urine of mice with a urinary tract infection produced by the injection of a Col^+ strain into a kidney (53). Infection produced by a colicin-sensitive strain injected into the other kidney was reduced, suggesting that the colicin retained its inhibitory activity in this environment. Colicin K-235 prevents experimental *Shigella* infections in guinea pig conjunctivae (543).

ECOLOGY OF OTHER BACTERIOCINS

The production of substances which inhibit the growth of closely related species seems to be almost universal among groups of bacteria. At the present time, there is little doubt that the colicins, with the possible exception of colicin M-K260 (*see* Relationships Between Colicins), are justifiably classified together and seems likely that they all have a similar role.

On turning to other groups of bacteria, and particularly to gram-positive genera, the inhibition zones seen on agar plates are more likely to be due to various unrelated inhibitors. The results of surveys which measure the incidence of "bacteriocinogeny" in bacteria other than members of the *Enterobacteriaceae* are particularly difficult to assess in view of the reports of bacteriocin-like action due to such diverse agents as ammonia (482), hydrogen peroxide (264, 389), lactic acid (567), phospholipases (including clostridial α-toxin) (114, 455), hemolysins (58, 259), the lytic enzyme lysostaphin (501), bacteriophage tails or defective bacteriophages (49, 142, 298, 308, 496), phospholipids, and fatty acids (580).

Nevertheless, there are substances produced by gram-positive bacteria which are analogous to colicins. But in view of the many types of inhibitors produced, definitions of bacteriocins are perhaps less useful than an assessment of how far they are analogous to colicins in terms of their structure, function, and evolution.

Although there are many analogies with colicins, several bacteriocins produced by gram-positive bacteria share features which are not found in the well-studied colicins. Several have molecular weights of about 10,000 and are not induced by mitomycin C, although many prophages in gram-positive bacteria are inducible. Many of them associate into higher-molecular-weight complexes. Indirect evidence, such as the loss of bacteriocinogeny after treatments which usually cure plasmids, suggests that many of the bacteriocins are almost certainly plasmid determined. A bacteriocin produced by *Clostridium perfringens* has been shown more directly to be plasmid determined (292) and it is also inducible (293). The bacteriocin/hemolysin produced by *Streptococcus faecalis* var. *zymogenes* and that produced by staphylococci of bacteriophage type 71 (group II) are also plasmid determined (301, 583).

Streptococcus

On the basis of Sephadex G-100 gel filtration, bacteriocins produced by strains of group A and group H streptococci were found to have molecular weights of about 8,000 and 29,000 (503, 548). Bacteriocins produced by a strain of *Streptococcus agalactiae* and a strain of *Lactobacillus helveticus* (LP27) had molecular weights of 10,000 and 12,400 (355, 570). All were inactivated by proteases. Lactocin 27 was isolated in the form of a non-dialyzable protein-lipopolysaccharide complex which was dissociated into glycoprotein subunits after treatment with SDS (570, 571). The bacteriocin isolated from a group H streptococcus was found to have a molecular weight of 29,000 at pH 10.8, but it associated into a higher-molecular-weight complex (molecular weight 110,000) in pH 7.6 buffer of lower ionic strength (503).

The transmission of bacteriocinogeny determined by group D streptococci by means of a process which was deoxyribonuclease resistant

suggests that these streptocins may be determined by self-transmissible plasmids, which would further strengthen the analogy between streptocins and colicins (301, 566).

Bearing in mind the difficulties of interpretation, the frequency of bacteriocin production by streptococci is often high, between 20 and 50% in several investigations. There are conflicting reports about the importance of streptocins in modifying the microbial flora in natural environments and there are indications that streptocins are inactivated, presumably by proteases, in dental plaque and saliva (325).

To determine whether bacteriocins may be important in the succession of strains in the throat, Crowe et al. (103) examined throat cultures from 40 children for 20 weeks. The results of this single study indicated that the throat flora of children which did not become colonized by group A streptococci comprised significantly more strains which inhibited the growth of group A streptococci in laboratory tests. The inhibitory bacteria were largely *Streptococcus viridans*, nonhemolytic streptococci, and strains of *Neisseria*. Although the differences between the two groups was statistically significant, many of the strains isolated from children who became colonized by group A streptococci inhibited this bacterium in laboratory tests. Of 144 of the strains from those who did not become colonized, 72 (50%) were inhibitory compared with 58 out of 187 (32%) of strains from those who became colonized.

The incidence of bacteriocinogeny among pathogenic streptococci has also been investigated, although in most cases it is not clear whether all the inhibition zones are due to streptocins. Kuttner (360) found that 32 group A streptococci isolated from cases of acute glomerulonephritis all inhibited an indicator group A strain on agar plates. Serological types normally associated with nephritis were also more likely to be inhibitory than were types of group A streptococci normally associated with other infections. However, Overturf et al. (454) did not find a correlation between nephritogenic type and inhibitory action on agar plates, although nephritogenic types produced larger, clearer inhibition zones.

Turning to group D streptococci, Montgomerie et al. (417) found a correlation between bacteriocinogeny and severity of infection produced experimentally in the rat kidney. The ability of four bacteriocinogenic and four non-bacteriocinogenic strains of *Streptococcus faecalis* to produce kidney infections was investigated. The number of bacteriocinogenic bacteria per gram of infected kidney was significantly greater than the number of non-bacteriocinogenic streptococci.

Enterocines produced by *Streptococcus faecalis* may also affect the course of infection by strains of *Clostridium* (215, 356). Many clostridia are inhibited by group D streptococci on agar plates (356) and a fivefold increase in minimum lethal dose of *Clostridium perfringens* (in guinea pigs) was observed when a mixed infection (intramuscular injection) with an inhibitory strain was made. The inhibitor produced by the streptococci was sensitive to proteases.

Brock et al. (59) found that more than 50% of enterococci produced inhibitory substances and classified them into five groups on the basis of such characteristics as sensitivity to chloroform, proteases, and heat. The bacteriocin from *Streptococcus faecalis* var. *zymogenes* strain X-14 illustrates the difficulties of defining a bacteriocin. The bacteriocin is active against a wide range of gram-positive bacteria and it is also a hemolysin. It is probably determined by a plasmid which is self-transmissible by conjugation (301, 566). Both the bactericidal and hemolytic activities are probably functions of the same protein; both activities were lost with similar kinetics at high temperature and were inhibited by lecithin (58). Granato and Jackson (204, 205, 206) demonstrated that the bacteriocin/hemolysin had two components: a small protein (molecular weight 11,000) possessed the lytic activity and another protein (molecular weight 27,000) activated the lytic protein. Whether these proteins have a primary role in their action on host cells or on other bacterial cells is unknown. It has been suggested that they might be involved in membrane or cell wall synthesis or in transport (206). A similar bacteriocin described by Bottone et al. (43) had a wider spectrum of action and was particularly effective against *Diplococcus pneumoniae*. The immunity of the producing strain to enterocin X-14 has little in common with immunity to colicin E3-CA38; immune cells produce a specific teichoic acid (19, 114, 204).

A more unusual property has been suggested for streptocin A produced by some *Streptococcus pyogenes* strains. Tagg and McGiven (546) suggest that it may be implicated in rheumatic fever through the damage it produces on heart muscle, allowing heart-reactive antibodies to reach underlying heart tissues. But there are reports that only about half of the strains isolated from cases of rheumatic fever inhibit other streptococci (360, 454, 470). Streptocin A has a molecular weight of about 8,000 and is sensitive to proteases (548). It inhibits macromolecular synthesis in bacterial cells and also brings about the degradation of RNA (547).

Staphylococcus

Several bacteriocins have been isolated from

strains of staphylococci. Each comprises protein together with lipid or lipid and carbohydrate and each dissociates into subunits with molecular weights of about 10,000 after SDS treatment of a higher-molecular-weight form. Staphylococcin synthesis is not induced by mitomycin C or UV and the bacteriocin remains largely cell bound.

Staphylococcin 1580 isolated from a strain of *Staphylococcus epidermidis* was found to be a protein-lipid-carbohydrate complex with a molecular weight of between 150,000 and 400,000 in polyacrylamide gels (311, 314); in SDS the bacteriocin dissociated into subunits (molecular weight about 15,000) each comprising about 42% protein (311). Staphylococcin 462 isolated from a strain of *Staphylococcus aureus* is a lipoprotein which was not released into the medium during growth in liquid cultures or after mechanical disruption (223, 224), but it was released from the cells treated with 7 M urea. A complexed form could be dissociated with SDS into subunits with a molecular weight of about 9,000 (223). Similarly, staphylococcin 414 isolated from a strain of *S. aureus* was found to be largely cell bound. A lipopolysaccharide complex could be dissociated into subunits which had a molecular weight of about 12,500, in comparison with protein markers, in polyacrylamide gel (181).

Instability of staphylococcin production, particularly after treatment with curing agents such as acridine orange or ethidium bromide, suggests that several staphylococcins are plasmid determined (107, 181, 223, 312, 313, 460, 582). Warren et al. (583) have recently shown that exfoliative toxin, responsible for the production of scalded-skin syndrome by strains of phage type 71, and the bacteriocin produced by this strain are both determined by a plasmid with a molecular weight of 33×10^6. The pathogenic phage type, 71, almost invariably produces a bacteriocin which is particularly effective against *Corynebacterium diphtheriae* (460), although a wide range of gram-positive bacteria are inhibited (105, 106). It inhibits the synthesis of protein and RNA and also brings about the degradation of RNA in sensitive strains of *Streptococcus* (101, 106). The rescue of cells, which have been treated with bacteriocin by trypsin indicates a further parallel with the action of colicins (108).

The therapeutic use of staphylococcin has been attempted (467) and staphylococcin 414 does not elicit neutralizing antibody so it may have some therapeutic value. (In 1909 Schiotz reported attempts, which apparently had some success, to cure diphtheria carriers by spraying suspensions of staphylococci into their throats [see 517].)

Bacterial antagonism between closely related species is probably an important factor in determining the bacterial composition of the skin, but there is little evidence on which to assess the relative importance of bacteriocins in this antagonism (601). The importance of "bacterial interference," irrespective of the cause, in determining the composition of the microbial flora of the skin is illustrated by the work of Shinefield et al. (516–519), who made extensive studies on the antagonism of *S. aureus* strain 502A towards other bacteria. This strain does not produce a bacteriocin but its presence inhibits the growth and colonization of other strains of staphylococci. By artificially colonizing newborn children with strain 502A, epidemics produced by other strains of *Staphylococcus* in nurseries have been successfully controlled (518, 519). The strain has also been used in the treatment of recurrent furunculosis in man (517). Bacterial interference can also be demonstrated between strain 502A and other staphylococci in chicken embryos and in experimental burns in animals (13, 479).

Pseudomonas

Substances which are termed "pyocins" may be low-molecular-weight proteins resembling colicins (267) or proteins which resemble bacteriophage tails, for example, pyocin R1 (316). Pyocin R1 and similar pyocins have immunological cross-reactions with temperate bacteriophages found in *Pseudomonas* (226, 268, 300). The low-molecular-weight pyocins may become associated with endotoxin, resembling the complexed form of colicin K-235 when it is isolated from uninduced cultures (138, 265).

Investigations have been made recently on the protection against infection by *Pseudomonas aeruginosa* brought about by prior injection with pyocin, or, more precisely, with partially purified preparations from *Pseudomonas* strains producing pyocin (220, 395, 509). Haas et al. (220) used the supernatant from a culture of pyocin-producing bacteria as a source of pyocin. An intraperitoneal injection of the supernatant protected mice against *Pseudomonas* infection. But protection was given only against *Pseudomonas* strains which were sensitive to pyocin. Twenty mice given an intraperitoneal injection of pyocin followed by an injection 24 h later of one of four different pyocin-sensitive strains (five mice for each strain) were not killed, but five mice subsequently challenged with a pyocin-resistant strain were all killed. Protection could also be demonstrated after 1 h; an immune response was presumably not responsible for protection at this stage although this may not be the case for more prolonged protection. It would be interesting to know whether purified

pyocin, as well as culture supernatants containing pyocin, protects mice against *Pseudomonas aeruginosa* infection.

Pasteurella

The correlation between pesticinogeny and virulence in *Pasteurella pestis* (62, 64) is probably due to the pesticin being determined by a plasmid which also determines characters necessary for pathogenicity, rather than to the pesticin itself being a toxin of some sort. Pesticin I is a mitomycin C-inducible protein (29) which has a molecular weight of 65,000 (277). It resembles several colicins in lacking cysteine (277). Loss of the ability to produce pesticin is accompanied by decreased virulence (62, 64). But the fibrinolysin and coagulase determinants are also invariably lost (25), suggesting that all three proteins are determined by the same plasmid.

Neisseria

Preliminary reports (357, 579) suggest that the presence of strains of *Neisseria meningitidis* which inhibit *Neisseria gonorrhoeae* may prevent the establishment of the gonococcus in man. The meningocins resemble other bacteriocins synthesized by gram-negative bacteria in being inducible by mitomycin C (331).

CONCLUDING REMARKS

The analogies with bacteriophages which originally led Gratia to investigate the parallels between lysogeny and the nature of the antibiotic produced by *E. coli* V still provide a useful framework for discussing several aspects of colicins. It remains to be seen however, whether further work on the synthesis and mechanism of action of colicins and on colicin immunity will strengthen these parallels at the molecular level.

ColB and ColV factors are clearly related to other F-like plasmids. The heteroduplexes formed between F and ColV-K94 raise interesting questions about the reasons for the distinctive pattern of discontinuous homology between these two plasmids; the heterologous segments may be sets of functionally coordinated genes derived from other genomes through recombination. Whatever the explanation, selection rather than mere random drift is likely to be the most important factor; it may not be possible to account for this pattern of homology until we know more about the functions of the homologous and heterologous segments.

The chemistry of colicins themselves provide few clues about the origins of this group of antibiotics, except for certain pairs of colicins, Ia and Ib, and E2 and E3, which have clearly evolved from common ancestors. But the colicins as a whole do not have similar amino acid compositions. The synthesis of different colicins may be controlled in a similar manner, however; all tested colicins are inducible. And the induced synthesis of colicins is so similar to λ prophage induction that the mechanisms underlying both events are probably the same.

At least two stages may be distinguished in the mechanism of action of colicins, although most is known about the initial stage of adsorption to receptors in the outer membrane. The subsequent chain of events which leads to cell death is known in most detail for colicin E3 and closely related cloacin DF13 both of which affect the bacterial ribosomes. The effects of several other colicins on membranes or on membrane preparations suggest that these colicins come into contact with the cytoplasmic membrane and affect its energized state. In determining how colicins are rearranged or transported after adsorption, it would be useful to know whether colicin receptors are located at special regions on the cell envelope where there are connections with the cytoplasmic membrane.

Colicinogeny is one of many factors which contribute to the relative competitiveness of *E. coli* strains. The roles of other bacteriocins are less clear. At one time or another almost all views have been expressed about the ecological importance of colicins, from their having no significance whatever to their being the most important element in determining the succession of strains in the intestine. More work is needed before we can assess more precisely the ecology of colicins in different environments. The significance of bacteriocins in the establishment of pathogens and in the prevention of infection deserves further study. The considerable advances made in the genetics and molecular biology of colicinogeny may help not only to increase our understanding of the ecological significance of colicins, but also to establish the relationships between colicinogenic bacteria and Col plasmids as units of selection.

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